AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Solid lipid nanoparticles for brain tumors therapy: State of the art and novel challenges

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/128166> since

Published version:

DOI:10.1016/S0079-6123(08)80011-8

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

**Provided for non-commercial research and educational use only.
Not for reproduction, distribution or commercial use.**

This chapter was originally published in the book *Nanoneuroscience and Nanoneuropharmacology*. The copy attached is provided by Elsevier for the author's benefit and for the benefit of the author's institution, for non-commercial research, and educational use. This includes without limitation use in instruction at your institution, distribution to specific colleagues, and providing a copy to your institution's administrator.

All other uses, reproduction and distribution, including without limitation commercial reprints, selling or licensing copies or access, or posting on open internet sites, your personal or institution's website or repository, are prohibited. For exceptions, permission may be sought for such use through Elsevier's permissions site at:

<http://www.elsevier.com/locate/permissionusematerial>

From Andrea M. Brioschi, Sara Calderoni, Gian Paolo Zara, Lorenzo Priano, Maria Rosa Gasco and Alessandro Mauro, Solid lipid nanoparticles for brain tumors therapy: state of the art and novel challenges.

In: Hari Shanker Sharma, editors, *Nanoneuroscience And Nanoneuropharmacology*.

Elsevier, 2009, p. 193

ISBN: 978-0-444-53431-6

© Copyright 2009 Elsevier B.V.

Elsevier.

CHAPTER 11

Solid lipid nanoparticles for brain tumors therapy: state of the art and novel challenges

Andrea M. Brioschi^{1,*}, Sara Calderoni¹, Gian Paolo Zara², Lorenzo Priano^{3,4}, Maria Rosa Gasco⁵ and Alessandro Mauro^{1,3}

¹*Department of Neurology and Laboratory of Clinical Neurobiology, Ospedale S. Giuseppe, Istituto Auxologico Italiano, IRCCS, Verbania, Italy*

²*Department of Anatomy Pharmacology Forensic Medicine, University of Torino, Torino, Italy*

³*Departement of Neurosciences, University of Torino, Torino, Italy*

⁴*Department of Neurology, Ospedale S. Giuseppe, Istituto Auxologico Italiano, IRCCS, Verbania, Italy*

⁵*Nanovector S.r.l., Torino, Italy*

Abstract: Malignant gliomas, despite aggressive multimodal therapies and adequate supportive care, still maintain poor prognosis. Solid lipid nanoparticles (SLN) are colloidal carriers that could be regarded as a highly flexible platform for brain tumor imaging and therapeutical purposes. In this chapter we will first describe brain tumors characteristics and conventional therapeutical approaches. In the subsequent sections, we will analyze SLN properties, effectiveness, and future perspectives in both imaging and targeted treatment of malignant gliomas.

Keywords: solid lipid nanoparticles; brain tumors; drug targeting; brain tumor imaging; cholesterylbutyrate; angiogenesis

Brain tumors

Brain tumors constitute a complex of heterogeneous clinico-pathological diseases, often characterized by poor prognosis and associated with low quality of life (Buckner et al., 2007; DeAngelis, 2001; Louis, Pomeroy, & Cairncross, 2002).

Central nervous system (CNS) tumors are classified by the World Health Organization

according to their presumed cell of origin as well as to their localization, histopathological appearance, and lineage markers (Louis et al., 2007).

Primary brain tumors show in the United States an average annual incidence rate of 14.4 per 100,000 persons (Fisher, Schwartzbaum, Wrensch, & Wiemels, 2007) and about half of them are histologically malignant, showing an annual gender incidence rate of 7.0/100,000 in men and 5.2/100,000 in women (Fisher et al., 2007).

Primary malignant brain tumors account for the first cause of death for solid tumors in children and the third cause of death for all cancer types in

* Corresponding author.
Phone No. +39 323 514337; Fax No. +39 323 514364
E-mail: a.brioschi@auxologico.it

adolescents and young adults (Buckner et al., 2007). Primary malignant CNS neoplasms show a relative survival probability at 2 years of 37.7% and at 5 years of 30.2% (Fisher et al., 2007). These epidemiological data clearly suggest that primary malignant brain tumors — despite a lower incidence rate compared to all cancers (about 2%) — display high morbidity and mortality rate and consequently they could be numbered among the most devastating human neoplastic diseases (Buckner et al., 2007).

Gliomas are the most common type among primary CNS tumors and account for an average annual incidence rate in the United States of 6.42/100,000 persons (Fisher et al., 2007). Gliomas include different histopathological entities: astrocytomas, oligodendrogliomas, mixed gliomas (a combination of oligodendroglial and astrocytic features), and ependimomas (Fisher et al., 2007; Norden & Wen, 2006). According to the degree of differentiation and anaplasia, gliomas could receive a histopathological grade that, in turn, strictly correlates with prognosis. High-grade gliomas (otherwise defined malignant gliomas), accounting for more than half of all gliomas in adults and for 78% of all primary malignant CNS tumors, include glioblastomas, anaplastic astrocytomas, anaplastic oligodendrogliomas, and anaplastic oligoastrocytomas. Survival time after diagnosis of malignant glioma depends on both the histological subtype and the age at onset (Fisher et al., 2007; Sathornsumetee, Rich, & Reardon, 2007). The 2-year relative survival probability value (according to the histology and age group at diagnosis) in the United States is variable: 1.4–29.8% for glioblastomas, 4.1–71.4% for anaplastic astrocytomas, 4.9–76.5% for anaplastic oligodendrogliomas, and 37.6–84.7% for mixed gliomas (Fisher et al., 2007).

Genetic factors and molecular markers were recently identified as prognostic indicators for malignant gliomas, in addition to previously known clinical, histological, and neuroradiological factors such as age and functional status at diagnosis, extent of surgical resection, degree of necrosis, and pre- and postsurgery tumor size. (Sathornsumetee et al., 2007).

Different glioma subtypes and grades exhibit a set of peculiar genetic alterations, mainly occurring in genes encoding proteins involved in signal transduction pathways and cell-cycle regulation of tumor initiation and progression. These genetic changes frequently involve growth factors that can be overexpressed (i.e., epidermal growth factor — EGF, platelet-derived growth factor — PDGF, fibroblast growth factor, ciliary neurotrophic factor), or show activating mutations like those commonly occurring (40% of glioblastomas) in the EGF receptor — EGFR gene. Other common molecular changes include tumor suppressor loss (i.e., Phosphatase and Tensin (PTEN) mutations, occurring in nearly 25% of glioblastomas) or deletions, *ink4a/arf* locus deletions, *Rb* and *p53* mutations (Cavaliere, Wen, & Schiff, 2007; Fisher et al., 2007; Fomchenko & Holland, 2006; Martin-Villalba, Okuducu, & von Deimling, 2008; Sanson, 2008).

For instance, altered EGFR expression inversely correlates to survival increasing proliferation rates, resistance to chemotherapy, invasion, and apoptosis (Rich & Bigner, 2004). Moreover, PDGF ligands are highly expressed in malignant gliomas and the activation of PDGF receptors stimulates proliferation, resistance to apoptosis, cellular motility, and angiogenesis (Rich & Bigner, 2004).

In addition to the aforementioned variability in the pattern of genetic alterations, during tumor progression glioma cells could display additional mutations and epigenetic changes that yield these tumors to become genetically and phenotypically different from the cancer-initiating focus and perhaps sharing variable levels of chemo- and/or radiosensitivity (Cavaliere et al., 2007; Fomchenko & Holland, 2006; Wong, Bendayan, Rauth, Li, & Wu, 2007). Among these latter additional changes we could mention the hypermethylation of methylguanine-DNA-methyltransferase (MGMT) promotor (that results in reduced MGMT expression and consequently in a better response to alkylating drugs) and contrariwise the possible development of a multidrug resistance phenotype by the activation of membrane-associated transporters (such as P-glycoprotein) that actively expel from the cytoplasm a broad range

of cytotoxic agents (Criniere et al., 2007; Martin-Villalba et al., 2008; Wong et al., 2007). In conclusion, gene expression profile could help to differentiate glioma subtypes in order to identify tumor indistinguishable on morphological ground (such as primary and secondary glioblastomas) and thereby to predict clinical course (Martin-Villalba et al., 2008).

Taken together, previously reported data suggest that malignant gliomas could be regarded as a group of different diseases, each of them showing distinctive clinical–pathological behavior (Fisher et al., 2007; Louis et al., 2002). Consequently, the recognition of specific prognostic factors may be crucial to identify different subgroup of patients who could be more sensitive to differing schedules of conventional treatment options (i.e., combining chemotherapeutics and/or radiotherapy with treatment sensitizers) (Cavaliere et al., 2007). Furthermore, the identification of these prognostic factors will open a new therapeutical way (the so-called molecular chemotherapy) both by using new treatment agents (including, i.e., monoclonal antibodies, cytokines, synthetic molecules, gene constructs) and by targeting different extra- and/or subcellular pathways (such as cell-cycle control, cell migration, tissue invasion, and angiogenesis) (Cavaliere et al., 2007; Sanson, 2008).

To date, despite aggressive multimodal therapeutic approaches (such as surgery, radiation therapy, and chemotherapy) and adequate supportive care, malignant gliomas still maintain poor prognosis. Among newly diagnosed glioblastoma patients that receive the best treatment schedule possible median survival rate is 14.6 months (Buckner et al., 2007; Carpentier, 2005; Fisher et al., 2007; Norden & Wen, 2006; Norden, Drappatz, & Wen, 2008a; Rich & Bigner, 2004; Stupp et al., 2005).

This substantial failure of conventional treatments could be ascribed to three main reasons, principally related to the peculiar characteristics of high-grade gliomas.

1. First of all, the inability to achieve effective intratumoral concentrations of common chemotherapeutic agents, mainly due to the presence of the blood–brain (BBB) and the

brain–tumor barrier, as well as to the intrinsic properties of commonly used cytotoxic drugs (i.e., poor specificity, high systemic toxicity, and propensity to induce chemoresistance).

2. Furthermore, the characteristic early infiltrative behavior of these neoplasms that limits surgical aggressive resections and thereby negatively influence multimodal approaches.
3. Finally, the noticeable cellular and genetic intratumoral, spatial–temporal heterogeneity that modifies the individual response to chemo- and radiotherapy (Cavaliere et al., 2007; Fomchenko & Holland, 2006; Sanson, 2008; Sanson, Laigle-Donadey, & Benouaich-Amiel, 2006).

The normal BBB is a highly effective physical and physiological barrier that regulates the CNS homeostasis and thereby controls the delivery of drugs to the brain (Blakeley, 2008; Kaur, Bhandari, Bhandari, & Kakkar, 2008). Mechanical limitations are mainly carried out by endothelial cell tight junctions that in turn are supported by the absence of fenestration and the reduction of pinocytotic vesicles at endothelial level and by the presence of a composite anatomical barrier constituted by astrocytic end-feet, pericytes, and extracellular matrix. Physiological properties that characterize the normal BBB are formed by the presence of both high electrical resistance across the endothelial cell barrier (even reaching $2,000 \omega \text{cm}^2$) and effective efflux transporters (mainly members of the adenosine triphosphate-binding cassette — ABC), located on cell surface of endothelial and cancer cells (Blakeley, 2008; Kaur et al., 2008; Pardridge, 2007; Wong et al., 2007).

Several factors influence the specific ability of a given molecule to pass through the BBB, including size, water solubility, charge, plasma protein binding, and serum concentration (Blakeley, 2008; Kaur et al., 2008). Other factors, such as cerebral blood flow rate, influx and efflux values at the BBB and blood–CSF (cerebrospinal fluid) barrier, rate of metabolism, and interactions–binding of the drug in the brain, may influence drug cerebral distribution (Kaur et al., 2008). However, only less

than 5% of all drugs proved active into CNS, and almost 100% of large molecule drugs — including recombinant proteins and enzymes, monoclonal antibodies, antisense agents, short interfering RNA, and gene products — under physiological conditions do not cross the BBB (Pardridge, 2007). Invasiveness and neoangiogenic processes of malignant gliomas are accompanied by focal disruptions of the BBB and increased permeability of capillary endothelium. Nevertheless, this BBB disruption is not able to produce any effect on tumor therapy response probably because both intrinsic characteristics of tumor cells (i.e., high proliferation rate and chemo- and radiotherapy escape phenomena) and the not homogeneous localization of these vascular breakages into the tumor mass (Beduneau, Saulnier, & Benoit, 2007).

Nowadays, the most common chemotherapeutic agents in clinical use for malignant glioma treatment include DNA-alkylating cytotoxic drugs (such as carmustine), triple combination (often used at high dosages) of procarbazine, cisplatin, and vincristine, and the more recently available temozolomide (TMZ), etoposide, and lomustine. Novel strategies to achieve effective intratumoral bioavailability of chemotherapeutic agents, regardless of their physical-chemical properties, partially escaping in a passive manner the BBB control, were proposed. Drug dose intensification, use of more lipophilic analogs, and intra-arterial delivery preceded by iatrogenic disruption of the BBB using osmotics, magnetic resonance imaging (MRI)-guided ultrasound, or radiotherapy showed debatable or uncertain results (Blakeley, 2008; Rich & Bigner, 2004). Postsurgical implantation into residual tumoral cavity of drug-embedded biodegradable polymers or catheters (for both convection-enhanced delivery or reservoir continuous release) are in clinical use but are still limited to a well clinically selected group of patients referring to much more selected neurosurgical teams (Beduneau et al., 2007; Blakeley, 2008; Kaur et al., 2008). Furthermore, active methods to cross the BBB are in study and will be evaluated in the following sections of this chapter.

Drug delivery systems — solid lipid nanoparticles

In order to obtain a better profile of drug stability, biodistribution, pharmacokinetics, and anticancer activity after parenteral administration, so allowing more targeted antitumoral activity, lower systemic toxicity and reduced adverse side effects, several passive and active carriers were developed. Among them, lipoplexes, dendrimers, cyclodextrins, liposomes, microspheres, niosomes, and nanoparticles were investigated in experimental models and some of them were even put on the market (Barratt, 2003; Cho, Wang, Nie, Chen, & Shin, 2008; Gaidamakova, Backer, & Backer, 2001; Kim et al., 2005; Koziara, Lockman, Allen, & Mumper, 2006; Kreuter, 2001; Lu et al., 2005; Mehnert & Mader, 2001; Muller & Keck, 2004; Olbrich, Bakowsky, Lehr, Muller, & Kneuer, 2001; Pardridge, 2007; Parveen & Sahoo, 2008; Rich & Bigner, 2004; Serikawa et al., 2006; Tiwari & Amiji, 2006; Wong et al., 2007).

An effective delivery system should display some of the following characteristics:

- ability to load a high amount of drugs,
- physical and chemical storage stability,
- low systemic toxicity (that is to say favorable *in vivo* fate of the carrier)
- easy and large-scale production process,
- low overall cost,
- chance of specifically target tumor tissue (Kaur et al., 2008; Mehnert & Mader, 2001).

Solid lipid nanoparticles (SLN) are colloidal (namely, submicron sized) carriers constituted by a solid lipid matrix at room and body temperature, composed of physiological lipids (lipid acids, mono-, di-, or triglycerides, glycerine mixtures, and waxes), and stabilized by biocompatible surfactants (nonionic or ionic) (Marcato & Duran, 2008; Wissing, Kayser, & Muller, 2004; Wong et al., 2007). SLN were shown to satisfy nearly all the aforementioned characteristics combining some advantages (mainly drug bioavailability, controlled release, and drug targeting) and avoiding disadvantages of other vehicles in a more simple and versatile way (Blasi, Giovagnoli, Schouben, Ricci, & Rossi, 2007; Kaur et al., 2008).

Fig. 1. Schematic structure of loaded SLN obtained by warm microemulsion method.

SLN could be prepared by different approaches such as high-pressure homogenization at high or low temperatures, warm microemulsions (Fig. 1), solvent emulsification–evaporation–diffusion, and high-speed stirring and/or sonication (Blasi et al., 2007; Muller, Mader, & Gohla, 2000). The first two processes show the most versatile technique (mainly in terms of avoidance of nonbiocompatible components, scale-up feasibility, and sterilization) and consequently are the most frequently used (Blasi et al., 2007).

SLN could carry different agents including both hydrophilic and lipophilic therapeutics and diagnostic tools. For parenteral administration benzodiazepines, antipsychotics, pilocarpine, steroids, timolol, antineoplastic agents, peptides, and more recently gene therapeutical agents, such as plasmid DNA and antisense oligonucleotides (AS-ODN), and MRI contrast agents were successfully incorporated into SLN (Dass, 2002; Gasco, 2007; Manjunath & Venkateswarlu, 2005; Muller et al., 2000; Peira et al., 2003; Wissing et al., 2004). Furthermore, SLN could be administered by different routes (such as parenteral, transdermal, oral, and ocular) (Gasco, 2007). The drug solubility in the lipid melt together with the structure and the polymorphic state of the lipid matrix are the main factors that influence the drug loading capacity (Wissing et al., 2004).

SLN are able to increase chemical stability and to protect from systemic degradation the vehiculated molecule (hence consequently increasing its plasma half life) by virtue of the presence of a solid hydrophobic core (the so-called solid high melting fat matrix) in which lipophilic compounds

are dissolved or dispersed (Kaur et al., 2008; Wissing et al., 2004). The carried drug — according to its lipid ratio and solubility — could be mainly located into the core, into the shell or dispersed into the matrix of the SLN (Wissing et al., 2004). By modifying the composition of the lipid matrix, the type and the concentration of the surfactant, and the productions parameters it is possible to modulate the drug release profile: drug-enriched shell (burst release), solid solution (intermediate release), and drug-enriched core (sustained release, even for up to several weeks) (Cavalli et al., 2003; Wissing et al., 2004).

The SLN content of only well-tolerated, biocompatible, and biodegradable lipids and the avoidance of any organic solvent during the preparation process justify the common statement that SLN could be regarded as safe (Blasi et al., 2007; Fundaro et al., 2000; Zara et al., 1999, 2002a, 2002b).

Moreover, SLN could be easily sterilized and produced on a large industrial scale, so reducing the overall cost (Blasi et al., 2007; Gasco, 2007; Kaur et al., 2008).

Several *in vivo* experimental studies demonstrated that pharmacokinetics and body distribution profile of different drugs after parenteral administration are significantly changed if vehiculated by SLN. Among the tested agents we could count different compounds for which the passage through the BBB is usually troublesome: chemotherapeutics (i.e., doxorubicin, paclitaxel, idarubicin, camptothecin, etoposide, retinoic acid, TMZ), chemosensitizers (such as verapamil and cyclosporine-A), neuroleptics (such as clozapine), and contrast agents for MRI. These studies (that will be better analyzed in the following section) clearly demonstrated that SLN are able to significantly increase plasma peak, modify plasma concentration curve (raising the area under curve — AUC — from 3- to 20-fold and lowering the rate of clearance so increasing plasma half-lives), and reduce the volume of distribution (Fundaro et al., 2000; Huang, Zhang, Bi, & Dou, 2008; Manjunath & Venkateswarlu, 2005; Shenoy, Vijay, & Murthy, 2005; Wissing et al., 2004; Wong et al., 2007; Yang et al., 1999; Zara et al., 1999, 2002a, 2002b). Furthermore, a different body

distribution pattern of the drug was shown: usually the highest concentrations and mean residence times (MRT) are found in the brain, the lowest ones are seen in lung, heart, and kidney, and variable results are obtained from liver and spleen (Fundaro et al., 2000; Huang et al., 2008; Manjunath & Venkateswarlu, 2005; Shenoy et al., 2005; Wissing et al., 2004; Yang et al., 1999; Zara et al., 1999, 2002a, 2002b). These properties, probably justified by the effect of SLN on both the rate of crossing biologic barriers and the pattern of drug release, coupled with the reduction of the drug total dose needed, could significantly contribute to decrease side effects of carried agents (Shenoy et al., 2005).

Compared to other vehicles, SLN show a higher ability to escape the reticuloendothelial system (RES), so bypassing liver and spleen filtration and consequently increasing the bioavailability of the carried agent (Cho et al., 2008; Kaur et al., 2008). SLN characteristics (mainly size and surface) could be easily modified in order to modulate the body distribution hence increasing bioavailability into CNS of the complex drug carrier. Size not exceeding a maximum diameter of 200 nm, sphericity, and adequate deformability are crucial peculiarities to ensure the escape from the sinusoidal spleens (Cho et al., 2008; Kaur et al., 2008). The coating of SLN surface with a hydrophilic or flexible polymer (such as polyethylene glycol, PEG) and/or the use of a surfactant (such as polysorbate and Epikuron) prevent opsonization [namely, the recognition by macrophage membrane of peculiar blood plasma proteins (opsonin) adsorbed onto the colloidal carrier] and the consequent phagocytosis carried out by macrophages in the liver (Cho et al., 2008; Kaur et al., 2008). This mechanism was summarized in the concept of the “differential protein adsorption” under that physical–chemical surface characteristics of nanoparticles induce qualitatively and quantitatively different adsorption patterns that in turn determine the *in vivo* fate of the carrier system (Muller & Keck, 2004). In addition to opsonins (mainly immunoglobulins and complement factors), that facilitate RES recognition, dysopsonins (such as albumin, apolipoprotein A-I, A-IV, C-III, and H) are contrariwise able to

reduce the affinity of colloidal carriers to RES and perhaps to increase passive targeting to specific organs only by modifying the composition of the nanoparticle (W. Mehnert & Mader, 2001; Muller & Keck, 2004).

The mechanisms by which SLN cross the BBB are not completely understood but it is indisputable that a central role is played by the interactions between plasma proteins adsorbed onto the SLN surface and endothelial cells, hence facilitating or hindering nanoparticles adhesion and subsequently activating or not endocytotic process (Goppert & Muller, 2005; Kreuter, 2001; W. Mehnert & Mader, 2001). Among proteins adsorbed onto the SLN surface, ApoE, Apo C-II, albumin, and immunoglobulin G seem to be crucial in the site-specific targeting to the brain (Blasi et al., 2007; Goppert & Muller, 2005). Other mechanisms, namely, increased retentions of nanoparticles in the brain blood capillaries and transcytosis, could be advocated and could work together with the aforementioned endocytotic process (Blasi et al., 2007).

Furthermore, different surfactants (such as Polysorbate 80 and Poloxamer 188) were shown to facilitate the BBB crossing of different drugs (i.e., doxorubicin) vehiculated by both polybutylcyanoacrylate (PBCA) nanoparticles and SLN (Blasi et al., 2007; Cho et al., 2008; Dehouck et al., 1997; Goppert & Muller, 2005; Kaur et al., 2008; Petri et al., 2007; Steiniger et al., 2004).

For instance, our group showed that *in vivo* SLN containing stearic acid and PEG 2000 as stealthing agents, unloaded or loaded with different chemotherapeutics (i.e., doxorubicin), are able to facilitate the passage through the BBB and to increase the bioavailability of the drug into the brain tissue compared to nonstealth SLN or free drug solutions; moreover, stealth SLN show lesser degree of recognition by the RES so prolonging drug plasma half-life (Fundaro et al., 2000; Podio, Zara, Carazzonet, Cavalli, & Gasco, 2000b; Zara et al., 2002b).

Yang and Colleagues showed that camptothecin-loaded SLN stabilized by Poloxamer 188 compared to the free solution of this antineoplastic agent after i.v. administration induce a higher maximum concentration (corresponding to 180%

increase) and a better profile of the AUC/dose curve and MRT in the brain, heart, and RES (Yang et al., 1999).

Koziara and Colleagues evaluated the CNS uptake of two kinds of SLN composed by the emulsifying wax (E wax) or Brij 72 as matrix, and, respectively, Brij 78 and Tween 80 as surfactant. The SLN were labeled with [^3H]cetyl-alcohol and the transport of the SLN was measured by an “*in situ*” rat brain perfusion method. A significant increase in the CNS uptake of both types of SLN was observed compared to [^{14}C]sucrose (Koziara, Lockman, Allen, & Mumper, 2003, 2004). The same group confirmed the aforementioned results in an analogous experiment using paclitaxel-loaded polysorbate nanoparticles (Koziara et al., 2004).

Petri and Colleagues recently showed in a *in vivo* rat intracranial glioblastoma model that both the surfactants Polysorbate 80 and Poloxamer 188 promote the adsorption onto PCBA-nanoparticles of various blood plasma proteins, including different classes of apolipoprotein (respectively Apo E and Apo A-I). These apolipoproteins in turn activate a specific receptor-mediated mechanism at the capillary brain endothelial cells: Polysorbate 80–Apo E complex activate a LDL-receptor mediated endocytosis and Poloxamer 188–Apo A-I stimulate a scavenger receptor class B type I (SR-BI)-mediated nanoparticle adhesion (Dehouck et al., 1997; Petri et al., 2007; Steiniger et al., 2004).

Moreover, the use of differently charged surfactants significantly influences the passage through the BBB. Lockman and Colleagues evaluated the effects of differently charged nanoparticles on both the BBB integrity and the brain permeability. The authors showed that only neutral and low concentrations of anionic nanoparticles warrant the BBB integrity and that the brain uptake is better for low concentration of anionic nanoparticles. These results suggest that neutral and low concentrations of anionic nanoparticles can be regarded as effective colloidal carriers to the brain (Lockman, Koziara, Mumper, & Allen, 2004).

In conclusion, the aforementioned data show that SLN could be effectively and easily tailored (mainly acting on the composition of their surface)

in order to passively increase CNS targeting (passive targeting). Furthermore, SLN are able to allow a more specific targeting directed to genetic and phenotypic features displayed by brain tumors, the so-called active targeting, that will be discussed in the following sections of this chapter (Parveen & Sahoo, 2008).

Solid lipid nanoparticles and brain tumors

As introductory remarks we have to remind that *in vitro* and *in vivo* experimental glioma models are not able to fully reproduce the extremely complex characteristics of human gliomas, both phenotypically and genotypically. More in details, *in vitro* models based on established primary animal or human glial tumor cell cultures (Barth, 1998; Claes et al., 2008; Fomchenko & Holland, 2006; Martinez-Murillo & Martinez, 2007; Mathieu, Lecomte, Tsanaclis, Larouche, & Fortin, 2007) are useful to study biochemical and biological tumor cell properties (such as chemo- and radiosensitivity) but cannot recapitulate the interactions between the tumor and the host environment (i.e., neoangiogenesis and immunological reactions) as well as the genetic variability of human gliomas. Furthermore, *in vivo* models (mainly subcutaneous or brain orthotopic xenografts of selected primary glioma cell cultures), although influenced by tumor–host interactions, do not show some distinctive phenotypical features of naïve malignant gliomas — such as diffuse infiltrative behavior or angiogenesis. Moreover, these models are lacking genetic heterogeneity and native stromal support as well as provide a synchronous instead of a stepwise disease development paradigm (Claes et al., 2008; Fomchenko & Holland, 2006). In conclusion, experimental glioma models are not able to accurately reproduce the naturally occurring disease and consequently the findings obtained from these models have to be critically and with careful consideration translated into clinical phase I and II trials and hence to clinical practice (Claes et al., 2008; Fomchenko & Holland, 2006).

In the following sections we will mainly focus on SLN prepared by our group from warm

microemulsions. Microemulsions are chemical-physical systems that are composed of oil, water, cosurfactant, and surfactant, and that show an interfacial tension near zero, thus accounting for their long-term stability. Microemulsion nanodroplets display a mean diameter below 80 nm. Warm microemulsions are prepared at temperature ranging from 60°C to 80°C by using melted lipids (such as fatty acids/triglycerides) and are subsequently dispersed in cold water. Nanodroplets obtained using this procedure become SLN, which are successively washed by tangential flow filtration. SLN display spherical shape and a narrow size distribution. The zeta potential is always high (30/40 mV), being negative or positive according to the starting formulation.

Drugs of different structure and lipophilicity, such as paclitaxel and doxorubicin, were loaded into SLN using different methods. Drug-loaded SLN show a mean diameter ranging from 80 to 200 nm, depending on the chemical characteristics and the amount of the incorporated molecules.

In vitro experimental models

Intracellular trafficking of nanoparticles

In previous sections of this chapter we already evaluated the properties that a systemically administered colloidal carrier must display in order to effectively reach brain tumor mass. At this level, the carrier should also display other abilities in order to pass through the selective plasma membrane and to reach effective concentrations in the cytoplasm of neoplastic cells as well as to display a controlled drug release.

In nonphagocytic cells, the preferential mechanism of nanocarriers cellular uptake is mediated by endocytosis (Rejman, Oberle, Zuhorn, & Hoekstra, 2004; Soldati & Schliwa, 2006). Endocytic pathway of nanoparticles starts at the plasma membrane level and it can be either clathrin-dependent or clathrin-independent, and the latter could be in turn divided into caveolar or clathrin-caveolae-independent (Mayor & Pagano, 2007). The first step of the clathrin-mediated endocytosis

is a receptor-mediated process based on ligand-receptor recognition and interaction at membrane level. Subsequently, the pathway progress by generating clathrin-coated pits that invaginate into the cytoplasm and then detach from it, so forming the endocytic vesicles. Therefore, these vesicles undergo to both early and late endosomal transport and lastly to lysosomal digestion. On the other hand, caveolin-coated vesicles invaginate from plasma membrane domains which are especially enriched in cholesterol and sphingolipids (Simons & Ikonen, 1997). This caveolae-mediated uptake could be very advantageous in carrier-mediated drug delivery because nanoparticles can avoid the lysosomal degradation, so increasing their cytoplasmic half-life and consequently maintain for longer periods a sustained intracellular drug release (Shin & Abraham, 2001). However, Lai and Colleagues suggested that certain types of polymeric nanoparticles can exploit a nonclathrin, noncaveolae, and cholesterol-independent pathway in order to undergo nondegradative trafficking into HeLa cells (Lai et al., 2007). Among other physiological mechanisms perhaps involved in nanocarrier intake spontaneous fluid-phase macropinocytosis could be included. This process, commonly occurring in all eukaryotic cells during their life time, is characterized by cyclic internalization of plasma membrane bits in which substances dissolved in the extracellular fluids are entrapped. Through this physiological recycling pathway it is possible that positively charged or neutral nanoparticles glued to the negative outer surface of the cell membrane could reach the cytoplasm (Partlow, Lanza, & Wickline, 2008).

One or more of the aforementioned pathways could be involved in neoplastic cellular uptake of nanoparticulate carriers, depending on their size, z-potential, chemical characteristics, and coated molecules (Cho et al., 2008; Edetsberger, Gaubitzer, Valic, Waigmann, & Kohler, 2005; Vasir & Labhasetwar, 2007; Vijayaraghavalu, Raghavan, & Labhasetwar, 2007).

Several studies demonstrated that SLN (including also both polymer-lipid hybrid nanoparticles and nanostructured lipid carriers) are able to easily enter into the cytoplasm of different tumoral cells including U373 (human astrocytoma), U87 MG

(human glioblastoma–astrocytoma), Lipari (human glioblastoma), and C6 (rat glioma) (Brioschi et al., 2009; Lim, Lee, & Kim, 2004; Miglietta, Cavalli, Bocca, Gabriel, & Gasco, 2000; Serpe et al., 2006; Stevens, Sekido, & Lee, 2004; Wong, Bendayan, Rauth, Wu, 2004; Wong et al., 2006a, 2006b). Nevertheless, the exact mechanism by which SLN cross the cell membrane is still now poorly understood (Muller & Olbrich, 1999). We observed (unpublished data) that fluorescent SLN are rapidly uptaken from human U373 tumoral cells (in 5 min) and accumulate into cytoplasm. Moreover, SLN do not enter either into the nucleus or into cytoplasmic organelles such as mitochondria or Golgi apparatus. Furthermore, after 4 h we observed a lysosomal entrapment of the greater part of the uptaken nanoparticles. The persistence of few SLN into the cytoplasm could lead us to suppose the existence of either alternative concomitant endocytotic entrance pathways or a mechanism for lysosomal escape. Moreover, SLN seem to display a biphasic drug release profile: from 10 to 30 min we observed a characteristic “burst release” phase while from 30 min to 24 h SLN produced a till robust and sustained drug delivery. These data seem to suggest that at early times, corresponding to the cytoplasmic localization, SLN rapidly release loaded compounds until an equilibrium with the environment is reached. Subsequently, the prolonged and till sustained drug delivery could be probably ascribed to lysosomal digestion of SLN lipid matrix by resident acidic lipases (Du, Sheriff, Bezerra, Leonova, & Grabowski, 1998).

Further studies will clarify both the role played by different endocytotic processes possibly involved in SLN tumor cell uptake and the contribute given by the lysosomal escape mechanism in the delayed drug release phase.

Antineoplastic drugs loaded into SLN

Several chemotherapeutics were incorporated in SLN, such as doxorubicin, idarubicin, paclitaxel, camptothecin, etoposide, SN-38 (irinotecan analog), retinoic acid, 5-fluorouracil (5-FU), and TMZ.

Anthracyclin antibiotics such as doxorubicin, idarubicin, and daunorubicin have general anticancer properties that include interaction with DNA in a variety of different ways such as intercalation, DNA strand breakage, and inhibition operated by topoisomerase II. Most of these compounds at effective dosages produce significant toxicity. Doxorubicin (Di Marco, 1978; Stan, Casares, Radu, Walter, & Brumeanu, 1999) is currently in clinical use for the treatment of several solid tumors, while daunorubicin and idarubicin are exclusively used for the treatment of leukemia. However, due to their chemical properties, anthracyclines do not easily pass the BBB and hence they do not achieve effective intracerebral concentrations for the treatment of brain tumors. Moreover, the dose-related characteristic systemic side effects such as cardiomyopathy, congestive heart failure (Minotti, Menna, Salvatorelli, Cairo, & Gianni, 2004; Singal, Li, Kumar, Danelisen, & Iliskovic, 2000), bone marrow depression, and alopecia (Minow, Benjamin, Lee, & Gottlieb, 1977) have to be taken into account.

Paclitaxel is a diterpenoid isolated from *Taxus brevifolia* that shows anticancer effects against both hematopoietic and solid tumors (von Holst et al., 1990). Because of its high hydrophilicity, paclitaxel does not easily cross the BBB. Moreover, its clinical use is highly limited by systemic side effects, such as peripheral neuropathy and cardiac arrhythmia (Rowinsky & Donehower, 1995) as well as alopecia and bone marrow depression (Minow et al., 1977).

Camptothecin, an alkaloid plant isolated from *Camptotheca acuminata* (Wall, Wani, Natschke, & Nicholas, 1986), is the prototype of antitumor agents that display a peculiar mechanism of action. These compounds target the nuclear enzyme topoisomerase I that physiologically transiently breaks and rejoins DNA strands in order to facilitate their replication, recombination, and transcription. Because of poor water solubility, instability at biological pH, and severe toxicity of the carboxylated form, camptothecin is not used in clinical applications (Potmesil, 1994).

Etoposide, a semisynthetic derivative of podophyllotoxin, a substance extracted from the mandrake root *Podophyllum peltatum*, shows potent

antineoplastic properties (Xu, Lv, & Tian, 2009). More in details, etoposide binds to and inhibits topoisomerase II main function of ligating cleaved DNA molecules and consequently induces accumulation of single- or double-strand DNA breaks, inhibition of DNA replication and transcription, and apoptotic cell death. Moreover, etoposide do not readily penetrate the CNS. In the first 24 h during i.v. administration of etoposide at various dosages, the concentration of this drug in the CSF ranges from undetectable to less than 5% of that concurrently found in the plasma.

SN-38, an irinotecan analog derived from camptothecin (O'Dwyer & Catalano, 2006), is a topoisomerase I inhibitor primarily used in the treatment of colorectal cancer.

Tretinoin, also known as all-*trans*-retinoic acid, is a natural derivative of vitamin A. Retinoids are important regulators of cell reproduction, proliferation, and differentiation and are commonly used to treat dermatological disorders (Cheepala, Syed, Trutschl, Cvek, & Clifford, 2007). Furthermore, tretinoin could also be regarded as a pro-differentiating antineoplastic agent and it is used in the treatment of acute promyelocytic leukemia (Cornic et al., 1992).

5-FU — an analog of uracil — is converted to a fraudulent nucleotide that impair thymidylate synthesis. The result is inhibition of DNA synthesis but not RNA or protein production (Cohen, Flaks, Barner, Loeb, & Lichtenstein, 1958).

TMZ is an orally available methylating agent at specific DNA sites and thus affects DNA synthesis and consequently triggers apoptosis. TMZ became a new standard-of-care treatment of patients affected by glioblastoma, both as adjuvant and as concurrent chemotherapy during radiotherapy (Friedman, Kerby, & Calvert, 2000; Sathornsumetee et al., 2007). This alkylating agent is able to pass through the BBB and to achieve in the CSF approximately 40% of the corresponding plasma concentration. Unfortunately, TMZ displays adverse effects such as hematological toxicity and oral ulceration and an unusual cardiomyopathy, directly due to the accumulation of the drug in the heart (Sathornsumetee et al., 2007).

Cytotoxicity of SLN loaded with chemotherapeutic agents

In vitro, the efficacy of doxorubicin- or paclitaxel-loaded SLN compared to drug-free solutions were evaluated in different neoplastic cells, including glioma and astrocytoma cell lines.

In a recent study our group compared the intracellular accumulation and toxicity in human tumoral cell lines (including U373 astrocytoma) of different doxorubicin formulations: loaded into SLN (Doxo-SLN), carried by pegylated liposomes (Caelyx), and administered as free solutions (Serpe et al., 2006). Doxo-SLN were significantly more efficient in inhibiting cell growth in comparison to both pegylated liposomes and free solutions, suggesting that the intrinsic characteristics of the delivery system by itself may improve the uptake and accumulation of doxorubicin into the cells.

Doxorubicin- and paclitaxel-SLN showed an improved cytotoxicity when compared to the free solutions at same concentrations; moreover, in human glioma cell lines (U87 and U373) drug-loaded SLN were able to induce consistent cell death at lower concentrations (from 10- to 100-fold) and at shorter exposure times if compared to drug-free solutions (Mauro & Brioschi, unpublished data, Miglietta et al., 2000).

Recently, polymer-lipid hybrid nanoparticle and lipid nanoparticles loaded with paclitaxel or doxorubicin and SLN loaded with vinorelbine bitartrate were prepared and their cytotoxicity was tested on tumoral cell lines of nonglial origin, showing promising results.

Wong and Colleagues developed a new polymer-hybrid nanoparticle system able to load and release water-soluble doxorubicin; they obtained a complex between cationic doxorubicin and soybean oil-based anionic polymer, dispersed together with a lipid in water to form Doxo-loaded SLN (Wong et al., 2006b). Treatment of Multidrug Resistant (MDR) cells (human breast cancer) with this formulation induced an increase of cell death when compared to the drug-free solution.

Stevens and Colleagues synthesized and incorporated paclitaxel-7-carbonyl-cholesterol, a paclitaxel prodrug, into lipid nanoparticles that also

contained folate-PEG-cholesterol as ligand for targeting folate receptor (FR) expressing tumoral cells (Stevens et al., 2004). The FR-targeted lipid nanoparticles showed greater uptake and cytotoxicity than the nontargeted ones in FR(+) cell lines (M109 and KB) than in FR(-) cell lines (CHO).

Wan and Colleagues evaluated the uptake and cytotoxicity of PEG 2000-stearic acid SLN loaded with vinorelbine bitartrate in RAW26 (mouse macrophages), MCF-7 (human breast cancer), and A549 (human alveolar basal epithelial) cell lines (Wan et al., 2008). They demonstrated that the phagocytic uptake of SLN by RAW26 are progressively inhibited by the addition of increasing concentrations PEG 2000; inversely, high quantities of PEG 2000 promote the intracellular uptake of SLN by tumoral cell lines such as MCF-7 and A549, in accordance with previously reported data (Bocca et al., 1998).

Moreover, the assay of anticancer activity *in vitro* demonstrated that, due to the increased cellular internalization of drug, the cytotoxicity of vinorelbine bitartrate is enhanced by encapsulation in pegylated SLN.

Jain and Colleagues prepared plain SLN and SLN loaded with 5-FU subsequently targeted with ferritin (Fr-SLN) using the ethanol injection method (S. K. Jain et al., 2008). The cellular uptake and IC_{50} values of the Fr-SLN formulation were determined *in vitro* in MDA-MB-468 breast cancer cells. *In vitro* cell binding of Fr-SLN exhibits 7.7-fold higher binding of Fr-SLN to cancer cells in comparison to SLN; moreover, cytotoxicity assays on Fr-SLN gave IC_{50} of 1.25 μ M and 3.56 μ M for plain SLN.

In vivo experimental models

SLN pharmacokinetics in healthy animals

Pharmacokinetics of SLN were first studied by us in healthy rats treated intravenously with Doxo-SLN or with the free drug solution (Zara et al., 1999). This study demonstrated the superior efficacy of SLN in achieving and maintaining doxorubicin plasma concentration in comparison to the

free solution. Moreover, SLN were able to modify the drug biodistribution, in particular, decreasing heart and liver drug concentration while improving cerebral accumulation.

Afterward, Fundarò and Colleagues compared doxo-SLN, stealth doxo-SLN and doxorubicin-free solution confirming the ability of SLN (stealth more than nonstealth) to increase plasma half-life and brain accumulation of doxorubicin (Fundaro et al., 2000). On the contrary, the free doxorubicin solution was very rapidly cleared from the blood stream (in 2.5 h) and is not able to enter the brain parenchyma. In all rat tissues examined, except the brain, the amount of doxorubicin was always lower after injection of the two types of SLN in comparison to the commercial solution; in particular, SLN significantly decreased heart concentrations, thus decreasing the characteristics side effects of the drug.

Furthermore, to confirm tissue distribution and transport across the BBB of modified SLN, both drug-free and drug-loaded stealth (pegylated) and nonstealth SLN were administered intravenously to rats (Podio, Zara, Carazzonet, Cavalli, & Gasco, 2000a). In the first part of the experiment, rats were injected with labeled (with 17- 131 I)doheptadecanoic acid) nonstealth or stealth SLN and radioactivity tissue accumulation measured after 60 min. This study showed that in liver and lungs the radioactivity was much lower after stealth-SLN formulation administration compared to the nonstealth counterpart, confirming that there is a difference in body distribution among the two SLN types, perhaps due to the stealth agents (stearic acid and PEG 2000). In the second part of this work, rats were injected with unlabeled stealth and nonstealth SLN and after 20' both types of SLN were detected in the brain thus confirming the BBB passage, as proved by CSF samples transmission electron microscopy analysis.

Finally, we demonstrated that pegylated doxorubicin SLN reach the brain in larger amounts than the nonstealth SLN and that the brain drug concentrations increase proportionally to the percentage of stealth agent used in the formulation (Zara et al., 2002b). Favorable pharmacokinetics and tissue distribution were demonstrated also for idarubicin-loaded SLN after i.v. or

duodenal administration routes (Zara et al., 2002a). SLN-based formulation was more effective in maintaining plasma drug concentrations than the idarubicin-free solution (improved AUC). Tissue distribution was significantly modified by the encapsulation: after SLN administration, idarubicin and idarubicinol concentrations are lower in heart, lung, spleen, and kidneys, while brain accumulation was enhanced. Duodenal route further on improved idarubicin pharmacokinetics compared to i.v. injection, thus suggesting that SLN can be considered for oral delivery of antineoplastic drugs in both systemic and brain tumors.

Yang and Colleagues evaluated the body distribution of intravenously injected camptothecin SLN (CA-SLN) in C57BL/6J mice (Yang et al., 1999). SLN were obtained from high-pressure homogenization technique using camptothecin, stearic acid, soybean lecithin and Poloxamer 188. The results of this study showed that the AUC/dose and the mean residence times of CA-SLN were much higher than those of camptothecin solutions, especially in brain, heart, and reticuloendothelial cells containing organs.

Chen and Colleagues by using the emulsification–evaporation technique, prepared stearic acid–lecithin SLN containing paclitaxel, coated with either Brij78 or Poloxamer F₆₈ surfactants (D. B. Chen, Yang, Lu, & Zhang, 2001). Evaluation of drug pharmacokinetics in Kunming (KM) mice showed that encapsulation of paclitaxel in both kind of SLN produce noticeable differences compared to the free drug (Cremophor EL) pharmacokinetics.

Huang and Colleagues prepared temozolomide SLN (TMZ-SLN) by emulsification and low-temperature solidification method (Huang et al., 2008). The AUC/dose and MRT of the TMZ-SLN i.v. injected in healthy rabbits demonstrated much higher and longer than those obtained with TMZ solution, especially in brain and in reticuloendothelial cells-containing organs; moreover the AUC ratio between the TMZ-SLN and TMZ solution in the brain was the highest among the tested organs.

Taken together, these studies demonstrate that SLN can modify the distribution of loaded drugs.

In particular, doxorubicin, when vehiculated by SLN, is able to achieve lower concentrations in lung, liver, and heart compared to the free solution, thus being able to reduce its systemic toxicity. At the same time, doxorubicin brain accumulation is greatly enhanced if carried by SLN, allowing cerebral targeting for drug delivery in brain tumors. Overall, the addition of stealth agents to SLN seems to improve the aforementioned properties, mainly by decreasing the recognition of SLN by the RES in liver and spleen, thus increasing drug–SLN plasma half-life.

SLN pharmacokinetics in rats bearing glioma cell subcutaneous or intracerebral orthotopic xenografts

In order to assess SLN-mediated drug delivery in an *in vivo* glioma model, we established in Wistar rats orthotopic intracerebral stereotactic C6 cell implants. At day 14, rats were intravenously injected with either doxo-SLN or doxorubicin-free solution. Doxo-SLN achieved intratumoral drug concentrations ranging from 12- (after 30 min) to 50-fold (after 24 h) higher compared to free solutions at same times. Furthermore, in the contralateral healthy hemisphere, only doxorubicin vehiculated by SLN was able to reach subtherapeutic concentrations, ranging from 3.2 (after 30 min) to 12 µg/g (after 24 h), compared to free drug solution (Mauro & Guido, unpublished data).

Williams and Colleagues showed that the SLN formulation of SN-38 is able to increase drug plasma half-life in nude mice bearing subcutaneously xenografted human HT29 cells, a model of chemoresistant colon adenocarcinoma (Williams et al., 2003).

Jain and Colleagues in the second *in vivo* part of the aforementioned study, treated i.v. nude Balb/c mice bearing MDA-MB-468 breast cancer cells subcutaneous xenografts with either 5-FU solution, plain 5-FU-SLN, or Fr-5-FU-SLN (S. K. Jain et al., 2008). The authors showed that administration of Fr-5-FU-SLN formulation results in effective reduction of tumor growth as compared

with free 5-FU and plain 5-FU-SLN (delay in tumor growth and increase in life span). Furthermore, Fr-5-FU-SLN allow an increased drug level in the tumor and decreased systemic drug accumulation as well as a reduced IC_{50} compared to both plain 5-FU-SLN and 5-FU solution.

Further suggestions on how nanoparticles work *in vivo* came from three recent studies.

Steininger and Colleagues used an experimental animal model based on intracerebral implanted 101/8 glioblastoma cells in rats (Steininger et al., 2004). Implanted rats were injected at days 2, 5, and 8 with the following formulations: blank PBCA nanoparticles coated with polysorbate 80 (NP+PS), doxorubicin in saline (DOX), doxorubicin in 1% polysorbate solution (DOX+PS), doxorubicin bound to NP (DOX-NP), or doxorubicin bound to NP coated with polysorbate (DOX-NP+PS). Rats treated with DOX-NP+PS showed a significantly higher survival times compared to all other groups and a 20% rate of long-term remission without any evidence of neurotoxicity.

Xu and Colleagues produced PEG-coated PBCA nanoparticles loaded with paclitaxel and targeted with transferrin (ATN, actively targetable nanoparticles) or not targeted (NTN, nonactively targeted nanoparticles) (Xu et al., 2005). Pharmacokinetics and biodistribution studies of ATN, NTN, and paclitaxel solution were performed in KM strain mice bearing S-180 tumor nodules of about 10 mm, while the evaluation of antitumor activity *in vivo* were done in S-180-bearing KM mice. The authors showed that ATN exhibited a markedly delay in blood clearance in mice and higher paclitaxel levels at 24 h after ATN injection compared to that obtained after free drug solution administration. The distribution profiles of ATN showed that after i.v injection, the tumor accumulation of paclitaxel increases with time, and its concentration at 6 h was about 4.8–2.1 fold higher than those from, respectively, free paclitaxel and NTN administration. A significant tumor regression was observed and complete tumor remission was evident in five out of nine KM mice treated i.v. with ATN.

Ambruosi and Colleagues investigated the biodistribution of blank [^{14}C]-PBCA uncoated

and coated with Polysorbate 80 as well as doxorubicin-loaded Polysorbate 80-coated [^{14}C]-PBCA in glioblastoma 101/8-bearing rats after i.v. injection (Ambruosi et al., 2006). The authors showed that the overcoating of [^{14}C]-PBCA-Polysorbate 80 decreased their concentrations in RES organs, while the addition of doxorubicin to the pegylated formulation counteracts the coating effects perhaps by increasing the positive charge of the particles and consequently by altering their adsorption properties both to plasma proteins and to other cells in the body. However, the accumulation of [^{14}C]-PBCA-Polysorbate 80 nanoparticles in the tumor site and in contralateral hemisphere of glioma-bearing rats demonstrated the efficacy of the enhanced permeability and retention effect on brain delivery of nanoparticles. Despite the reduced rate of BBB passage displayed by doxorubicin-loaded Polysorbate 80-coated nanoparticles (perhaps due to the interaction between the drug itself and the surfactant), the concentration of doxorubicin at the tumor site was still higher than in contralateral hemisphere and in brains from healthy rats.

Taken together, the aforementioned results clearly showed that nanoparticles, and in particular SLN, are able to significantly increase intracellular and intratumoral bioavailability of various chemotherapeutics potentially highly effective for brain tumors. Furthermore, in comparison to free solutions, SLN allow a noteworthy reduction in the amount of incorporated drug required to produce cytotoxic effects (as showed by the significant reduction of IC_{50}). In this manner, drug-related and dose-dependent systemic side effects could be avoided.

New therapeutical strategies

Prodrugs, solid lipid nanoparticles, and brain tumors

The use of prodrugs was proposed to overcome pharmacokinetics limitations of otherwise potentially effective drugs. Till now, the decreased cytotoxicity rate, the increased serum opsonization

(limiting the passage through the BBB), and the reduced mobility within the brain of the new synthesized lipophilic analogs compared to the referring drugs were advocated as the cause of the disappointing results of prodrugs treatment of brain tumors (Blakeley, 2008; J. X. Wang, Sun, & Zhang, 2002).

Wang and Colleagues synthesized from the cytotoxic agent 5-fluoro-2'-deoxyuridine (FUDR) a lipophilic prodrug, the 3',5'-dioctanoyl-5-fluoro-2'-deoxyuridine (DO-FUDR), that in turn was incorporated into SLN prepared by thin-layer ultrasonication technique (DO-FUDR-SLN) (J. X. Wang et al., 2002). A comparative study in mice showed that DO-FUDR-SLN allow the best AUC–time curve, MRT and $t_{1/2}$ value in brain tissue compared to both DO-FUDR and free FUDR. More in details, the brain AUC–time curve of DO-FUDR-SLN was 2.06-fold higher than that of DO-FUDR and both these curves were, respectively, 10.97- and 5.32-fold higher compared to free FUDR. Furthermore, the brain $t_{1/2}$ value of DO-FUDR-SLN was 1.49-fold higher than that DO-FUDR. The overall drug targeting efficiency (TE^C) of DO-FUDR-SLN to the brain was about threefold higher compared to free FUDR solutions (respectively 29.84 and 11.77). Moreover, the TE^C of DO-FUDR-SLN was decreased in the hearth and kidney compared FUDR free solutions. These data clearly suggest that SLN are able to further enhance the brain targeting of even lipophilic prodrugs and perhaps to partially reduce systemic undesired effects.

Our group chose cholesterylbutyrate (Chol-but) (Fig. 2) — the ester of cholesterol and butyric acid — as another matrix to prepare, from warm oil-in-water microemulsions, Chol-but SLN as a prodrug of butyric acid (Bach Knudsen, Serena, Canibe, & Juntunen, 2003; Brioschi, Zara, Calderoni, Gasco, & Mauro, 2008). This molecule belongs to the family of short-chain fatty acids, physiological compounds produced in the colon of all mammalian organisms (Bach Knudsen et al., 2003; Miller, 2004; Santini, Gozzini, Scapini, Grossi, & Rossi Ferrini, 2001), and could be regarded as a prototype of an effective *in vitro* anti-inflammatory and anticancer drug whose clinical use is heavily limited by its poor

Fig. 2. Structure of cholesterylbutyrate.

pharmacokinetics (Egorin, Yuan, Sentz, Plaisance, & Eiseman, 1999; Miller, 2004; Pouillart, 1998).

Butyrate acts as an anticancer agent by inhibiting proliferation, by stimulating differentiation, and by inducing apoptosis in a wide panel of neoplastic cell lines (including colorectal, breast, gastric, lung, pancreas, and brain districts) (J. S. Chen, Faller, & Spanjaard, 2003; Miller, 2004; Santini et al., 2001). Butyrate could be also numbered as an endogenous member of the family of histone deacetylases (HDAC) inhibitors (HDACI). Disequilibrium in the balance between histone acetyltransferases and HDAC and altered expression of HDAC are involved in the development and the progression of cancer (Balakin, Ivanenkov, Kiselyov, & Tkachenko, 2007; Bolden, Peart, & Johnstone, 2006; J. S. Chen et al., 2003; J. M. Mehnert & Kelly, 2007). HDACI acts as antineoplastic agents by increasing acetylation of both nuclear histones and nonhistone proteins, so inducing transcriptional and nontranscriptional effects and consequently gene expression modulation and activation–inhibition of different pathways (Balakin et al., 2007; Entin-Meer et al., 2005; J. M. Mehnert & Kelly, 2007; Minucci & Pelicci, 2006). HDACI anticancer activities could also include the regulation of the host immune responses and tumor angiogenesis (Bhalla, 2005; Bolden et al., 2006) as well as — in particular for butyrate — mRNA stabilization and direct action on gene transcription (Jiang & Sharfstein, 2008; Lee, Kim, Kummer, Giaccone, & Trepel, 2008; Miller, 2004). Butyrate displays *in vitro* a broad and diversified antineoplastic activity, partially similar to other HDACI, suggesting a possible use of this drug as an effective alternative and/or synergic chemotherapeutic agent. However, *in vivo* studies

on butyrate were disappointing (J. S. Chen et al., 2003; Conley et al., 1998; Miller, Kurschel, Osieka, & Schmidt, 1987; Miller, 2004; Patnaik et al., 2002; Pouillart, 1998; Santini et al., 2001), mainly because of poor pharmacokinetics (such as rapid plasma clearance and high liver first pass metabolism) and adverse events (Miller et al., 1987; Pouillart, 1998; Patnaik et al., 2002; Santini et al., 2001; Chen, Faller, & Spanjaard, 2003; Miller, 2004; Conley et al., 1998). For these reasons, more stable and safer prodrugs of butyrate, such as acyloxymethyl esters (tributyrin, AN-1, AN-9), were developed (Entin-Meer et al., 2007; Nudelman et al., 2001; Reid et al., 2004; Rephaeli et al., 2006).

Antineoplastic effects of Chol-but SLN were analyzed *in vitro* on several cancer cell lines (Pellizzaro et al., 1999; Salomone et al., 2000; Serpe et al., 2004; Ugazio et al., 2001) and compared to sodium-butyrate (Na-but).

In nonsmall-cell lung carcinoma cell line (NIH-H460) cultures Pellizzaro and Colleagues showed that Chol-but SLN are able to induce 90% cell growth inhibition at concentration six times lower than Na-but. Complete growth inhibition was obtained at a concentration (0.25 mM) at which Na-but causes only about 55% growth reduction (Pellizzaro et al., 1999; Salomone et al., 2000; Ugazio et al., 2001).

In melanoma cell lines (human MELTO1 and mouse B16) Salomone and Colleagues found that Chol-but SLN compared to Na-but exert antiproliferative and proapoptotic effects at lower doses and shorter treatment times. Furthermore, these effects of Chol-but SLN are time- and dose-dependent within the first 24 h, whereas at prolonged times they become strictly dose-dependent. Moreover, a significant decrease of proliferating cells and an increase of cells blocked in the G0/G1 to S transition phase were seen after 24 h of Chol-but SLN treatment (Salomone et al., 2000).

In three human leukemic cell lines (Jurkat from lymphoid, U937, and HL-60 from myeloid origin) Serpe and Colleagues confirmed that Chol-but SLN (0.25, 0.5, and 1 mM) compared to Na-but (same concentrations) are able to induce a greater cell growth inhibition. Furthermore, the authors showed that *c-myc* expression is rapidly and

transiently downregulated in all the three cell lines after Chol-but SLN treatment (0.25 mM) whilst it is slightly decreased only in U937 cells after Na-but treatment at higher concentrations (1 mM). Cell-cycle arrest caused by Chol-but SLN is different among the two groups of cells: block in G1 phase for myeloid (U937 and HL-60) and mainly in G2 phase for lymphoid cells (Jurkat). This result could suggest a different mechanism of action of Chol-but SLN in the various cell types (Serpe et al., 2004).

Antineoplastic effects of Chol-but SLN were analyzed *in vitro* on several cancer cell lines and compared to Na-but, showing that Chol-but SLN exert cell growth inhibition and proapoptotic at lower doses and shorter treatment times in all the cell lines tested (Figs. 3 and 4). However, in these studies the effect of Chol-but SLN on the cell cycle of the various cell lines appeared different, suggesting that the mechanisms of the anti-neoplastic Chol-but SLN effects may be differently modulated in different cellular contexts (Serpe et al., 2004).

Moreover, in a pilot study we i.v. treated Wistar rats bearing intracerebral stereotactic C6 cell implants with Chol-but SLN 30 mg/kg or with saline every day from day 15th to 21st after implant and then sacrificed. Morphological and immunohistochemical analyses showed a significant shrinkage in tumors of treated animals. The implanted area was replaced by large cysts surrounded by residual tumor cells mostly displaying apoptotic or monstrous (multinucleated) features. Confocal microscopy studies showed that Fluorescent Chol-but SLN, labeled with 6-coumarin were rapidly internalized into tumor cells and persisted for few days into their cytoplasm.

In summary, our *in vitro* studies on different neoplastic cell lines and preliminary *in vivo* study in a rat glioma model convincingly indicate that Chol-but SLN are able to induce consistent antiproliferative and proapoptotic effects earlier and at significantly lower concentrations compared to Na-but. The mechanisms of action of Chol-but SLN and of butyrate are similar but do not completely overlap. For instance, after Chol-but SLN treatment, a significant increase in G2/M block compared to Na-but is observed in some, but not

Fig. 3. Cytotoxic activity analysis Methylthiazolyldiphenyl-tetrazolium bromide (MTT test) performed on four human glioma cell cultures (U87, U373, Lipari, DF) treated with Na-but and Chol-but SLN at different concentrations after 72 h (reproduced from Brioschi et al., Molecules, 2008).

Fig. 4. Flow cytometry DNA analysis performed on U373 human glioma cell cultures treated with Chol-but SLN 0.125 mM after 24 (left) and 48 (right) h (reproduced from Brioschi et al., Molecules, 2008).

in all, of cancer cell lines and the percentage of apoptotic cells are found higher, even at later times (unpublished data).

Therefore, Chol-but SLN could be regarded as suitable and highly effective prodrug of butyric acid, still maintaining chemical-physical,

pharmacokinetics, and pharmacodynamic properties of other SLN formulations.

Chol-but SLN seem to act as other nonselective HDACI (Peart et al., 2005) by modulating in a nonspecific manner different pathways, mainly involved in cell survival and proliferation. In malignant gliomas, Chol-but SLN, that proved to modify spontaneous immune response, could act also as a nonspecific stimulating agent of an already present but weak host immune reaction (Carpentier & Meng, 2006).

According to previously reported *in vivo* data, Chol-but SLN are able to effectively reach the CNS and thereby the implanted tumors, and at the same time to achieve significantly lower concentrations in other organs, hence decreasing systemic toxicity.

Antiangiogenic agents, solid lipid nanoparticles, and brain tumors

Histological hallmarks of malignant gliomas include extensive neovascularization. Among CNS neoplasms primary malignant gliomas show the highest new vessel formation rate that is strictly connected to aggressive clinical behavior (Birner et al., 2003; R. K. Jain et al., 2007; Johansson, Brannstrom, Bergenheim, & Henriksson, 2002; Lamszus et al., 2003; Mischel et al., 2003; Rong, Durden, Van Meir, & Brat, 2006; Toi, Matsumoto, & Bando, 2001; Zhou, Tan, Hess, & Yung, 2003). Among the different mechanisms recruiting new blood vessels in brain tumors, neoangiogenesis is regarded as the major player because of its direct correlation with tumor progression and hence with prognosis. Endothelial proliferations within newly sprouted vessels (a hallmark of human glioblastomas) is probably a direct effect of central tumor hypoxia and necrosis that in turn induce pseudopalisading cells secreting proangiogenic factors (Rong et al., 2006).

Vascular endothelial growth factor A (VEGF-A) and its receptor VEGFR2 are considered crucial players among several known angiogenic cytokines (Ferrara & Davis-Smyth, 1997; R. K. Jain et al., 2007; Ke, Shi, Im, Chen, & Yung, 2000; Ke, Shi, & Yung, 2002; Lamszus et al., 2003; Rong et al., 2006).

The VEGF family consists of 34- to 45-kDa dimeric glycosylated protein isoforms. VEGF165 — the predominant isoform — is produced in most normal tissues, including the brain, and in both low- and high-grade gliomas in which its expression rate directly correlates to grading, vascularity, clinical behavior, and inversely to prognosis (Ferrara & Davis-Smyth, 1997; Ferrara, Gerber, & LeCouter, 2003; Jansen, de Witt Hamer, Witmer, Troost, & van Noorden, 2004; Rong et al., 2006; Rosenstein & Krum, 2004a; Toi et al., 2001).

In brain tumors, VEGF-A expression is mainly and independently regulated by both hypoxia (through the hypoxia-inducible factor-1 α , HIF-1 α) and acidosis. In addition, different oncogenes and tumor suppressor genes, hormones, cytokines, and signaling molecules are able to modify VEGF expression pattern (R. K. Jain et al., 2007). Furthermore, not only malignant cells but also various host cells (such as stromal cells) and extracellular matrix could express VEGF in response to toxic insults.

Several experimental attempts to turn off the HIF/VEGF signaling pathway using different class of drugs and genes constructs were successful *in vitro* and *in vivo* to reduce tumor progression and angiogenesis. Various compounds such as angiostatin, anti-VEGF and anti-VEGF receptor (VEGFR) antibodies, inhibitors of VEGFR-2 tyrosine kinase activity, ribozymes, AS-ODN, and small interfering RNA constructs were tested to interfere with the VEGF signaling pathway (Breyer et al., 2000; Farhadi, Capelle, Erber, Ullrich, & Vajkoczy, 2005; Jansen et al., 2004; Kim et al., 2005; Lamszus et al., 2003; Niola et al., 2006; Peoch et al., 2002; Rich & Bigner, 2004). For instance, several clinical studies indicated that treatment of recurrent glioblastomas patients with a combination of bevacizumab (anti-VEGF humanized monoclonal antibody), various chemotherapeutics (i.e., TMZ, irinotecan) and radiotherapy significantly increases progression-free survival and reduces the need for steroids (Norden et al., 2008a; Norden, Drappatz, & Wen 2008b). Till now, different VEGFR inhibitors are under phase I and II clinical study (Norden et al., 2008a).

However, these approaches showed significant limits in the ability of overcome systemic degradation, reach effective bioavailability in the tumor target, and avoid systemic toxicity. Common adverse effects, such as hypertension, proteinuria, and increased risk of thromboembolism and hemorrhage, and the evidence that about 50% of patients develop antiangiogenic therapy resistance and time-variable response further on limit the clinical use of these class of therapeutics (Norden et al., 2008b). These drawbacks could be firstly related to both the physical-chemical properties of the drugs and the routes of administration (Barratt, 2003; Jansen et al., 2004; Koziara et al., 2006; Muller & Keck, 2004; Pardridge, 2007; Rich & Bigner, 2004; Tiwari & Amiji, 2006).

Furthermore, recent preclinical studies surprisingly showed that the blocking of VEGF-mediated neoangiogenesis could promote both tumor infiltration (perhaps by overexpression of proinvasive molecules or by co-option of existing cerebral blood vessels) and recruitment of circulating endothelial cells into the neoplasm (Norden et al., 2008a, 2008b). These data clearly suggest that at this moment anti-VEGF signaling pathway inhibition could optimally work only if combined to other cytotoxic chemotherapeutics, to non-VEGF-mediated antiangiogenic factors, or to radiotherapy (Norden et al., 2008a, 2008b).

We specifically designed — always from warm oil-in-water microemulsions — SLN vehiculating VEGF antisense oligonucleotides (VEGF-AS-ODN SLN) in order to downregulate VEGF expression in a rat glioma model. We studied the effectiveness of VEGF-AS-ODN SLN both *in vitro* in C6 glioma cell cultures under hypoxic conditions (Fukumura et al., 2001; Serganova et al., 2004; Tan et al., 2005), and *in vivo* in the intracerebral rat C6 glioma model (Brioschi et al., 2009).

In vitro, rat C6 glioma cells under both normal and hypoxic conditions were treated with VEGF phosphorothioate AS-ODN, either free or vehiculated by SLN for 24 and 48 h. VEGF phosphorothioate sense-ODN (S-ODN) as free solution and carried by SLN (VEGF-S-ODN SLN) as well as Fluorescent SLN (Flu-SLN) carrying 6-coumarin instead of ODN were also tested as control.

At confocal microscopy observation within 5 min after treatment with Flu-SLN a sharp and homogeneous cytoplasmic green fluorescence reached the maximum and persisted unchanged for almost 90 min. Western blot analysis of cell homogenates from untreated normoxic cultures depicted a pattern of VEGF expression similar to that found in rat normal heart and brain homogenates when compared to that of human controls. Under hypoxia cotreatment with VEGF-AS-ODN, VEGF-S-ODN or VEGF-S-ODN SLN did not produce any appreciable VEGF expression modulation at both 24 and 48 h. VEGF 120, VEGF164, and VEGF188 expression at 24 and 48 h increased under hypoxia, as expected, while progressively decreased after VEGF-AS-ODN SLN treatment. A statistically significant reduction ($p < 0.01$) was evident for all the VEGF isoforms after 48 h when compared not only to the hypoxic but also to the basal conditions (Fig. 5).

In experiments *in vivo* with the Wistar rat C6 glioma model, the implanted rats were randomized into four main groups, each one treated for three consecutive days with free VEGF-S-ODN, free VEGF-AS-ODN, VEGF-S-ODN SLN or VEGF-AS-ODN SLN, at different concentrations. Three days after treatment all the animals were sacrificed. In control animals, tumor cells, mainly in the perinecrotic areas and tumor borders, showed a clear cytoplasmic VEGF immunostaining. Interestingly, a similar VEGF immunoreactivity was found in hippocampal neurons as well as in large pyramidal cortical and cerebellar Purkinje neurons in both the hemispheres.

In animals treated with VEGF-S-ODN- and VEGF-AS-ODN-free solutions as well as with VEGF-S-ODN SLN any appreciable modification in the VEGF expression in both tumor and normal brain tissue was found. Only treatment with VEGF-AS-ODN SLN induced a great reduction of VEGF expression in both central and peripheral regions of the tumors. VEGF expression was also decreased in normal brain tissue, but to a lesser extent than in tumors (Fig. 6).

In summary, *in vitro* findings clearly indicate that our SLN allow highly effective, quick, and sustained ODN delivery into tumor cells (at least 500-fold more efficient than the free solutions).

Fig. 5. VEGF expression statistical analysis and semiquantification of western blot data from cell homogenates (reproduced with permission from Brioschi et al., Journal of Nanoneuroscience, 2009).

Similar results have been previously described by Tondelli and Colleagues using *c-myc* AS-ODN incorporated in polymeric nanospheres (Saleh, Stacker, & Wilks, 1996; Tondelli, Ricca, Laus, Lelli, & Citro, 1998). Taken together these data demonstrate that SLN could be regarded as a good carrier not only for chemotherapeutic drugs but also for gene therapeutical agents. Furthermore, *in vivo* study showed that VEGF AS-ODN SLN efficiently downregulate VEGF expression in neoplastic cells, effectively reaching every part of the implanted tumors (Brioschi et al., 2009).

Future perspectives and novel challenges

SLN compared to other colloidal carriers display more versatile structural properties and hence could be potentially modified in order to vehiculate simultaneously more than one therapeutical compound. This goal could be reached acting on both the preparation process and lipid composition as well as surfactants and cosurfactants use. In this manner it will be possible to design SLN able to carry two or more therapeutical agents having different molecular structure and physical-chemical characteristics. In addition to lipophilic molecules it could be supposed that more hydrophilic and/or ionic compounds could be simultaneously loaded

into SLN. Furthermore, SLN may be planned to allow a different release profile of the carried drugs, for instance by acting on the preferential location of the dispersed molecule into the core or into the shell portion of the nanoparticle. This could lead to time specific release profile of each carried drug. These statements suggest a future possible scenario in which a single carrier could be adapted to different requirements. For instance, SLN could be tailored to the clinical course of the disease, and constructed in order to act as “sensitizer” or preparatory to other therapies (i.e., surgery and radiotherapy), to take into account the genetic temporal and spatial heterogeneity of the tumor, and to reciprocally enhance the effects of the vehiculated drugs otherwise individually poorly effective.

Targeting to the brain

In the previous part of this chapter we already showed that SLN could be passively targeted to the brain by modifying both lipid composition and production processes. This passive targeting could be also sustained by the so-called enhanced permeability and retention effect, commonly found in systemic solid neoplasms (Parveen & Sahoo, 2008; Wong et al., 2007). The not homogeneous BBB disruption coupled with the secretion of vascular

Fig. 6. VEGF immunohistochemistry on xenografted tumor sections from: control rats (a), corresponding negative (without primary antibody) control rats (b), animals treated with 2 mg/kg AS-ODN-free solution (c), and 2 mg/kg AS-ODN-SLN (d). VEGF immunohistochemistry on brain sections (hippocampus) from AS-ODN (e)- and AS-ODN-SLN (f)-treated animals (reproduced with permission from Brioschi et al., *Journal of Nanoneuroscience*, 2009).

mediators facilitating extravasation and the raised pressure exerted by tumor mass and surrounding edema contribute to slow the drainage of macromolecules and to facilitate the accumulation within the tumor of particulate carriers within the tumor (Parveen & Sahoo, 2008; Wong et al., 2007).

In addition to passive processes, active targeting is one of the most promising and potentially effective results of the use of nanoparticulate carriers. Active targeting could facilitate the SLN transport into brain tumors but also increase the specificity of the drug delivery into a peculiar neoplastic cell

population. This could consequently further reduce the total amount of the drug effectively needed (and hence the possible systemic toxicity) and allow a better selected and temporal defined antineoplastic effect. Active targeting implies that SLN surface is suitably designed to specifically recognize peculiar tissues or cancer cells. Furthermore, active targeting to brain tumor cells may facilitate the BBB passage (Beduneau et al., 2007) addressing different influx–efflux transport systems displayed by brain endothelial cells that include

carrier-mediated transport (i.e., D-glucose), receptor-mediated endocytosis [such as insulin, insulin-like growth factor, folic acid, and transferrin (Tf)], and adsorptive-mediated endocytosis (Beduneau et al., 2007; Pardridge, 2007). Therefore, active targeting of nanoparticulate carriers to brain tumor cells could be achieved by three main mechanisms: ligand–receptor interaction, antibody–antigen recognition, and use of aptamers [namely, DNA or RNA fragments that bind with high affinity and specificity molecular intracellular and/or membrane-bound targets (Parveen & Sahoo, 2008)]. All these processes implies that different classes of targeted compounds are displayed by SLN surface by covalent or notcovalent linkages (Beduneau et al., 2007).

Ligand–receptor interaction

Tf receptor is 2- to 10-fold overexpressed in most of the tumor cells compared to normal cells and hence could be regarded as the prototype of potential targets useful in order to enhance carrier–tumor cell interaction. Furthermore, Tf receptors are also expressed on the luminal membrane of brain endothelial cells and through receptor-mediated endocytosis allow the internalization of iron-saturated Tf (Fishman, Rubin, Handrahan, Connor, & Fine, 1987; Gupta, Jain, & Jain, 2007; Qian, Li, Sun, & Ho, 2002). This result, that seems to suggest the effectiveness of active BBB crossing, was disputed because of the demonstration of Tf retroendocytosis after dissociation from the iron, the latter compound being the only one completely transcytosed by endothelial cells (Beduneau et al., 2007). However, Gupta and Colleagues developed SLN conjugated with transferrin (Tf-SLN) and loaded with the antimalarial quinine dihydrochloride in order to increase the delivery of these agents to the brain. The authors found that Tf-SLN show the lowest plasma concentration and the highest brain uptake compared to nonconjugated SLN and free drug solution (Gupta et al., 2007). FR could be regarded as another possible useful system to actively target brain tumor cells, because of its high expression rate at the level of

both endothelial and brain tumor cells (Beduneau et al., 2007). Stevens and Colleagues showed that paclitaxel prodrug-loaded SLN conjugated to folic acid are effective in mice bearing subcutaneously engrafted murine lung carcinoma cell tumors (Stevens et al., 2004).

Antibody–antigen recognition

Receptor-specific peptidomimetic monoclonal antibodies (MAb) could act as “molecular Trojan horse” and allow BBB crossing by any given attached compound. Till now, various types of so-called Trojan horse liposomes proved effective in brain targeting (Pardridge, 2007).

Tsutsui and Colleagues designed bionanocapsules to specifically *in vitro* and *in vivo* target human glioma cells. These nanoparticles, composed of antibody directed against human EGFR-VIII, were specifically uptaken by human Gli36 cells in culture and *in vivo* in a mouse orthotopic Gli36 intracerebral glioma model by direct intratumoral injection (Tsutsui et al., 2007).

Yang and Colleagues showed that a boronated monoclonal antibody directed against the EGFR-VIII linked to polyamido amine dendrimers is effective in a rat glioblastoma model (Yang et al., 2006b, 2008). This report confirms previous preclinical studies showing that monoclonal antibodies directed against the extracellular portion of EGFR-VIII and PDGFR could be effective in the treatment of gliomas and hence could be used to specifically target SLN carrying different drugs to glioma cells (Rich & Bigner, 2004). Similarly antibodies directed toward different target involved in the glioma VEGF signaling pathway (i.e., VEGFR) could be used to target SLN to glioma endothelial cells possibly interfering with the angiogenic process (Rich & Bigner, 2004).

Nevertheless, both ligand–receptor and antibody–antigen recognition could interact and activate systemic and local host biological reactions, potentially interfering with physiological antitumoral activities, such as immunological response. For this reason MAb directed to sites of the targeted molecule not involved in the endogenous ligand recognition were developed (Beduneau

et al., 2007). MAb-conjugated liposomes, also known as immunoliposomes, proved effective as brain drug delivery systems. For instance, Zhang and Colleagues developed immunoliposomes, carrying a plasmid DNA encoding the EGF receptor antisense mRNA, conjugated with two MAb directed to mouse Tf receptor (in order to pass through the BBB) and to human insulin receptor (in order to intratumor cell delivery). This study showed that these immunoliposomes are effective after i.v. administration in mice bearing U87 brain tumors (Zhang, Jeong Lee, Boado, & Pardridge, 2002). A similar result was obtained in the same brain glioma model using immunoliposomes carrying a short hairpin RNA targeting EGFR mRNA (Boado, 2005; Zhang et al., 2004). Till now, various colloidal carriers (including pegylated nanoparticles and NLC) conjugated with a murine MAb antirat Tf (OX26) are under study and show promising results as brain drug delivery systems (Beduneau et al., 2007; Pardridge, 2007).

Aptamers

Aptamers (namely, DNA or RNA oligonucleotides targeted to specific antigens) display some advantages over antibodies such as lower immunogenicity, higher target specificity and affinity, and better tissue penetration. Poly(lactide-co-glycolide) (PLGA) nanoparticles carrying docetaxel functionalized with an RNA aptamer recognizing a plasma membrane-specific antigen were successfully tested in both *in vitro* and *in vivo* models of prostate cancer (Farokhzad et al., 2006a; Farokhzad, Karp, & Langer, 2006b; Martin-Villalba et al., 2008).

In conclusion, taken together these studies clearly suggest that SLN — due to their versatility — could be easily and variously engineered in order to successfully achieve active targeting to malignant brain tumors.

Gene therapy of brain tumors

Malignant gliomas display high genetic heterogeneity, as previously discussed ((62 Martin-Villalba, A. 2008)). In the recent years, many researches

focused on the role of microRNAs (miRNAs) expression profile in diagnosis, staging, progression, prognosis, and response to therapy in brain tumors (Nicoloso & Calin, 2008). The miRNAs together with short interfering RNA belong to the family of small regulatory RNAs that act as riboregulators and are the crucial mediators of RNA interference strategy (Liu, Fortin, & Mourelatos, 2008). The miRNAs — namely, 19–25 nucleotides in length noncoding RNA — act at posttranscriptional level and are able to regulate gene expression by reducing the amount of transcribed mRNA and/or translated proteins (Liu et al., 2008). Consequently, alterations in miRNAs play a critical role in tumor initiation, progression, and metastasis (Liu et al., 2008) and, in fact, glioblastomas display an miRNAs expression pattern different from normal brain tissue (Ciafre et al., 2005; le Sage et al., 2007; Liu et al., 2008). MiR-21 is highly expressed (from 5- to 100-fold) in human glioblastomas and seems to act by interfering with the transcription of critical proapoptotic genes, probably reducing PTEN protein expression (Chan, Krichevsky, & Kosik, 2005). Furthermore, the cluster miR-221-222 is involved in cell-cycle regulation by targeting the CDK inhibitor p27^{kip1} (le Sage et al., 2007; Visone et al., 2007). These findings suggest that miRNAs could represent potential targets in brain tumor therapy and different agents such as modified antisense single-stranded oligonucleotide complementary to specific miRNA (LNAs anti-miRNAs) and chemically modified and cholesterol-conjugated single-stranded RNA complementary to a given miRNA (antagomirs) are under study in order to achieve targeted miRNA inhibition (Nicoloso & Calin, 2008). However, antagomirs showed the same limitations displayed by other therapeutical compound in order to reach the CNS bypassing the BBB: these agents were not able to play any action in the brain when systemically administered in mice while they proved effective if directly injected into the mouse cortex (Krutzfeldt et al., 2007).

We believe that SLN could be suitable carriers for RNA interference approach as well as, in broader terms, for gene therapy of brain tumors, as proved for instance by our work on VEGF AS-ODN previously reported. Moreover, more

specific and downstream or upstream located players belonging to various pathways involved in glioma initiation and progression, including neoangiogenesis, could be effectively targeted by using SLN. Nevertheless, till now active targeting did not show satisfactory results in clinical trials and antiangiogenic therapy proved significant effects only if combined with chemotherapy (Sanson, 2008; Vredenburgh et al., 2007). For instance, EGFR targeted inhibition in recurrent malignant gliomas proved dependent by the concomitant switching-off of the downstream PI3K/AkT pathway (Mellinghoff et al., 2005). However, clinical trials addressing both EGFR and PI3K/AkT (so called “vertical targeting”) showed disappointing results, probably because of the concurrent activation of multiple tyrosine kinase pathways (Sanson, 2008). Therefore, the concomitant targeting (“horizontal targeting”) of more than one tyrosine kinase receptors activated in malignant gliomas could be effective (Sanson, 2008; Stommel et al., 2007). Once more, SLN could offer a resourceful colloidal carrier platform for both vertical and horizontal targeted therapies of malignant gliomas.

Brain tumors imaging and thermotherapy

Till now, several types of nanoparticles targeted mainly to vascular epitopes (such as magnetic particles, quantum dots, immunotargeted nanoshells, liposomes, and dendrimers) have been developed for systemic cancer detection in order to increase both intratumor retention times and contrast enhancement. The use of these nanoparticles could allow not only earlier detection and prolonged observation times of the neoplastic lesions but also — according to the targeting molecules employed — more detailed phenotypical and/or genetic characterization of detected tumors (Parveen & Sahoo, 2008).

Superparamagnetic nanoparticles were developed as MRI contrast agents in order to increase the selectivity and detection abilities of brain tumor imaging (Chertok, David, Huang, & Yang, 2007; Muldoon, Sandor, Pinkston, & Neuwelt, 2005; Reddy et al., 2006). Different magnetic

nanoparticles composed of a magnetic core (usually iron oxide) produce a MRI visible hypointense signal drop out on T2-weighted images (negative contrast) due to their ability to strongly enhance proton spin–spin relaxation (Y. X. Wang, Hussain, & Krestin, 2001). Polyacrylamide nanoparticles encapsulating iron oxide proved excellent tumor contrast enhancement after i.v. administration to rats bearing intracranial 9L gliomas (Moffat et al., 2003; Reddy et al., 2006).

Our group studied the pharmacokinetics and biodistribution of SLN loaded with superparamagnetic iron oxides (SLN-Fe^A and SLN-Fe^B) compared to Endorem[®] as contrast agents for MRI in rats. After parenteral administration both types of SLN-Fe showed a slower blood clearance and a more prolonged CNS retention time (up to 135') compared to the commercial MRI contrast agent Endorem[®] (Peira et al., 2003).

Thermal ablation (namely, thermotherapy) often combined to chemotherapy could be potentially effective in the treatment of malignant gliomas but is highly limited by its nonfocalized field of action (Jain, 2007). Recent studies proved that both in animal glioma models and in selected patients (Maier-Hauff et al., 2007) the injection of magnetic nanoparticles into the tumors followed by exposure to an alternating magnetic field allows to a prolongation of survival, induces regression of tumor growth and is well tolerated. Moreover, several studies showed that magnetic nanoparticles, due to their magnetic responsiveness, could be retained at tumor sites for longer times after local application of external magnetic field (Chertok et al., 2007). Chertok and Colleagues recently demonstrated that — in rats bearing intracerebral orthotopic 9L-gliosarcoma tumors — the concomitant application of brain targeting magnetic field during i.v. administration of iron oxide nanoparticles induces a fivefold increase in tumor exposure to these nanoparticles compared to nontargeted neoplasms and a 3.6-fold rise in selective nanoparticles accumulation in tumors than in normal brain tissue (Chertok et al., 2007).

Taken together these data suggest that SLN — either specifically coated with glioma tumor-targeting agents or brain targeted using magnetic field exposure — could work as innovative tools in the field of brain

tumors imaging by improving sensibility and specificity of commonly used techniques. Moreover, the feasibility of loading these tumor-targeted imaging-devoted SLN with different classes of antineoplastic agents could argue their possible use as neuroimaging and concomitantly therapeutic carriers, opening the way of a selective, localized, and concomitant or subsequent thermo- and chemotherapy.

Thereby the prolonged retention time allows long-lasting observation time of the tumor behavior and hence could be useful in noninvasive *in vivo* MRI monitoring of therapeutical effects produced by SLN-carried drugs.

Future SLN therapeutical applications

Furthermore, SLN could be used to enhance the effects of new attractive targeted therapeutical strategies of brain tumors, such as boron neutron capture therapy (BNCT) and photodynamic therapy (Jain, 2007).

- BNCT could allow highly localized radiotherapy, possibly limited to a range of a single neoplastic cell, by producing a nuclear reaction between thermal neutrons and ^{10}B , leading to generation of α particles and ^7Li nuclei (Jain, 2007). As previously reported, boronated antibodies directed against to specific glioma targets could be effective if conjugated to different carriers (Yang et al., 2006a). Thereby, it will be possible to design SLN carrying on their surface glioma targeted boronated antibodies and this could open the way to highly localized cotreatments. For instance, by including into these SLN different antineoplastic agents (i.e., radiosensitizers, chemotherapeutics, gene constructs) we could obtain concomitant or subsequent potentiation of localized radiotherapy.
- The use of a photosensitizer (such as Photofrin[®]) for photodynamic therapy is based on the ability of this class of compounds to generate, after exposure to a specific wavelength light, toxic oxygen species into target cells. This therapy is greatly limited by systemic (and in particular cutaneous) side effects (Jain, 2007; Parveen & Sahoo, 2008). Reddy and Colleagues

incorporated into a polymeric nanoparticle targeted to tumoral neovasculature both Photofrin[®] and iron oxide, showing that, compared PDT delivered after treatment with either Photofrin[®] or nontargeted nanoparticles alone, these nanoparticles were more effective in prolonging survival in a rat intracerebral glioma model after Photodynamic Therapy (PDT) (Reddy et al., 2006). Better MRI resolution obtained by the use of these targeted nanoparticles vehiculating iron oxide could contribute to improve intracranial localization of the tumor and hence to facilitate PDT administration. Further on, superparamagnetic SLN could be successfully used for PDT if adequately coated with glioma tumor-targeting agent and loaded also with a photosensitizer molecule.

Conclusions

In the previous sections of this chapter we showed that SLN — due to their versatile properties — could be regarded as efficacious colloidal carriers for different classes of agents useful in both imaging and treatment of malignant gliomas. SLN allow the effective employment of otherwise toxic (and hence poorly efficacious at safer dosages) chemotherapeutics and could efficaciously vehiculate molecules having different chemical and ionic structure, including gene constructs, and acting on distinct pathways involved in tumor initiation–progression. Furthermore, these nanoparticles could be designed to escape the RES and thereby to passively target the brain, so increasing the AUC curve rate and prolonging the exposition time. Moreover, active targeting as well as the possibility of simultaneously vehiculate more than one compound and the feasibility of different release profiles are more than an attractive realistic perspective. These latter SLN properties could further on contribute to increase the drug selectivity contemporaneously reducing systemic toxicity. In summary, SLN could work as a highly flexible platform for brain tumor imaging and therapeutical purposes, allowing a more tailored approach to both

genetically–phenotypically distinct malignant gliomas and patients' stratification.

Nevertheless, our data clearly showed that even the most passively brain targeted SLN at the lowest effective concentration used reach every part of the body where they could release the vehiculated drug. Furthermore, once crossed the BBB, nonactively targeted SLN carry the therapeutical agent in every CNS region where, on the contrary, it could be undesirable and/or dangerous. As previously described, VEGF-AS-ODN SLN can reduce VEGF expression not only in glioma cells but also in hippocampal neurons, potentially interfering with protective and repair processes involving VEGF (Krum & Rosenstein, 1998; Rosenstein & Krum, 2004b; Yano et al., 2005; Yasuhara et al., 2005).

This intra-CNS low selectivity claims on the one hand further *in vivo* studies directed to identify the minimal effective drug dose needed and on the other hand the development of more selective active-targeted SLN, in order to minimize undesired effects in normal brain but also in healthy systemic tissues.

Despite these latter suggested possible limitations anyway it would be advisable to plan well designed and controlled phase I and phase II clinical trials in humans with SLN carrying antiglioma drugs.

Acknowledgments

This chapter is in memory of our beloved friends and colleagues Cinzia Miscio and Simonetta D'Alisa.

References

- Ambruosi, A., Khalansky, A. S., Yamamoto, H., Gelperina, S. E., Begley, D. J., & Kreuter, J. (2006). Biodistribution of polysorbate 80-coated doxorubicin-loaded [14C]-poly(butyl cyanoacrylate) nanoparticles after intravenous administration to glioblastoma-bearing rats. *Journal of Drug Targeting*, 14(2), 97–105.
- Bach Knudsen, K. E., Serena, A., Canibe, N., & Juntunen, K. S. (2003). New insight into butyrate metabolism. *The Proceedings of the Nutrition Society*, 62(1), 81–86.
- Balakin, K. V., Ivanenkov, Y. A., Kiselyov, A. S., & Tkachenko, S. E. (2007). Histone deacetylase inhibitors in cancer therapy: Latest developments, trends and medicinal chemistry perspective. *Anti-Cancer Agents in Medicinal Chemistry*, 7(5), 576–592.
- Barratt, G. (2003). Colloidal drug carriers: Achievements and perspectives. *Cellular and Molecular Life Sciences*, 60(1), 21–37.
- Barth, R. F. (1998). Rat brain tumor models in experimental neuro-oncology: The 9L, C6, T9, F98, RG2 (D74), RT-2 and CNS-1 gliomas. *Journal of Neuro-Oncology*, 36(1), 91–102.
- Beduneau, A., Saulnier, P., & Benoit, J. P. (2007). Active targeting of brain tumors using nanocarriers. *Biomaterials*, 28(33), 4947–4967.
- Bhalla, K. N. (2005). Epigenetic and chromatin modifiers as targeted therapy of hematologic malignancies. *Journal of Clinical Oncology*, 23(17), 3971–3993.
- Birner, P., Piribauer, M., Fischer, I., Gatterbauer, B., Marosi, C., Ambros, P. F., et al. (2003). Vascular patterns in glioblastoma influence clinical outcome and associate with variable expression of angiogenic proteins: Evidence for distinct angiogenic subtypes. *Brain Pathology*, 13(2), 133–143.
- Blakeley, J. (2008). Drug delivery to brain tumors. *Current Neurology and Neuroscience Reports*, 8(3), 235–241.
- Blasi, P., Giovagnoli, S., Schoubben, A., Ricci, M., & Rossi, C. (2007). Solid lipid nanoparticles for targeted brain drug delivery. *Advanced Drug Delivery Reviews*, 59(6), 454–477.
- Boado, R. J. (2005). RNA interference and nonviral targeted gene therapy of experimental brain cancer. *NeuroRx*, 2(1), 139–150.
- Bocca, C., Caputo, O., Cavalli, R., Gabriel, L., Miglietta, A., & Gasco, M. R. (1998). Phagocytic uptake of fluorescent stealth and non-stealth solid lipid nanoparticles. *International Journal of Pharmaceutics*, 175, 185–193.
- Bolden, J. E., Peart, M. J., & Johnstone, R. W. (2006). Anticancer activities of histone deacetylase inhibitors. *Nature Reviews. Drug Discovery*, 5(9), 769–784.
- Breyer, R., Hussein, S., Radu, D. L., Putz, K. M., Gunia, S., Hecker, H., et al. (2000). Disruption of intracerebral progression of C6 rat glioblastoma by *in vivo* treatment with anti-CD44 monoclonal antibody. *Journal of Neurosurgery*, 92(1), 140–149.
- Brioschi, A., Zara, G. P., Calderoni, S., Gasco, M. R., & Mauro, A. (2008). Cholesterylbutyrate solid lipid nanoparticles as a butyric acid prodrug. *Molecules*, 13(2), 230–254.
- Brioschi, A. M., Calderoni, S., Pradotto, L. G., Guido, M., Strada, A., Zenga, F., et al. (2009). Solid lipid nanoparticles carrying oligonucleotides inhibit vascular endothelial growth factor expression in rat glioma models. *Journal of Nanoneuroscience*, 1(1), 65–74.
- Buckner, J. C., Brown, P. D., O'Neill, B. P., Meyer, F. B., Wetmore, C. J., & Uhm, J. H. (2007). Central nervous system tumors. *Mayo Clinic Proceedings*, 82(10), 1271–1286.
- Carpentier, A. F. (2005). Neuro-oncology: The growing role of chemotherapy in glioma. *Lancet Neurology*, 4(1), 4–5.
- Carpentier, A. F., & Meng, Y. (2006). Recent advances in immunotherapy for human glioma. *Current Opinion in Oncology*, 18(6), 631–636.
- Cavaliere, R., Wen, P. Y., & Schiff, D. (2007). Novel therapies for malignant gliomas. *Neurologic Clinics*, 25(4), 1141–1171.

- Cavalli, R., Bargoni, A., Podio, V., Muntoni, E., Zara, G. P., & Gasco, M. R. (2003). Duodenal administration of solid lipid nanoparticles loaded with different percentages of tobramycin. *Journal Pharmaceutical Sciences*, 92(5), 1085–1094.
- Chan, J. A., Krichevsky, A. M., & Kosik, K. S. (2005). Micro-RNA-21 is an antiapoptotic factor in human glioblastoma cells. *Cancer Research*, 65(14), 6029–6033.
- Cheepala, S. B., Syed, Z., Trutschl, M., Cvek, U., & Clifford, J. L. (2007). Retinoids and skin: Microarrays shed new light on chemopreventive action of all-trans retinoic acid. *Molecular Carcinogenesis*, 46(8), 634–639.
- Chen, D. B., Yang, T. Z., Lu, W. L., & Zhang, Q. (2001). In vitro and in vivo study of two types of long-circulating solid lipid nanoparticles containing paclitaxel. *Chemical & Pharmaceutical Bulletin (Tokyo)*, 49(11), 1444–1447.
- Chen, J. S., Faller, D. V., & Spanjaard, R. A. (2003). Short-chain fatty acid inhibitors of histone deacetylases: Promising anticancer therapeutics? *Current Cancer Drug Targets*, 3(3), 219–236.
- Chertok, B., David, A. E., Huang, Y., & Yang, V. C. (2007). Glioma selectivity of magnetically targeted nanoparticles: A role of abnormal tumor hydrodynamics. *Journal of Controlled Release*, 122(3), 315–323.
- Cho, K., Wang, X., Nie, S., Chen, Z. G., & Shin, D. M. (2008). Therapeutic nanoparticles for drug delivery in cancer. *Clinical Cancer Research*, 14(5), 1310–1316.
- Ciafre, S. A., Galarzi, S., Mangiola, A., Ferracin, M., Liu, C. G., Sabatino, G., et al. (2005). Extensive modulation of a set of microRNAs in primary glioblastoma. *Biochemical and Biophysical Research Communications*, 334(4), 1351–1358.
- Claes, A., Schuurin, J., Boots-Sprenger, S., Hendriks-Cornelissen, S., Dekkers, M., van der Kogel, A. J., et al. (2008). Phenotypic and genotypic characterization of orthotopic human glioma models and its relevance for the study of anti-glioma therapy. *Brain Pathology*, 18(3), 423–433.
- Cohen, S. S., Flaks, J. G., Barner, H. D., Loeb, M. R., & Lichtenstein, J. (1958). The mode of action of 5-fluorouracil and its derivatives. *Proceedings of the National Academy of Sciences, USA*, 44(10), 1004–1012.
- Conley, B. A., Egorin, M. J., Tait, N., Rosen, D. M., Sausville, E. A., Dover, G., et al. (1998). Phase I study of the orally administered butyrate prodrug, tributyrin, in patients with solid tumors. *Clinical Cancer Research*, 4(3), 629–634.
- Cornic, M., Guidez, F., Delva, L., Agadir, A., Degos, L., & Chomienne, C. (1992). Mechanism of action of retinoids in a new therapeutic approach to acute promyelocytic leukemia. *Bulletin du Cancer*, 79(7), 697–704.
- Criniere, E., Kaloshi, G., Laigle-Donadey, F., Lejeune, J., Auger, N., Benouaich-Amiel, A., et al. (2007). MGMT prognostic impact on glioblastoma is dependent on therapeutic modalities. *Journal of Neuro-Oncology*, 83(2), 173–179.
- Dass, C. R. (2002). Vehicles for oligonucleotide delivery to tumours. *The Journal of Pharmacy and Pharmacology*, 54(1), 3–27.
- DeAngelis, L. M. (2001). Brain tumors. *The New England Journal of Medicine*, 344(2), 114–123.
- Dehouck, B., Fenart, L., Dehouck, M. P., Pierce, A., Torpier, G., & Cecchelli, R. (1997). A new function for the LDL receptor: Transcytosis of LDL across the blood-brain barrier. *The Journal of Cell Biology*, 138(4), 877–889.
- Di Marco, A. (1978). Mechanism of action and mechanism of resistance to antineoplastic agents that bind to DNA. *Antibiotics and Chemotherapy*, 23, 216–227.
- Du, H., Sheriff, S., Bezerra, J., Leonova, T., & Grabowski, G. A. (1998). Molecular and enzymatic analyses of lysosomal acid lipase in cholesteryl ester storage disease. *Molecular Genetics and Metabolism*, 64(2), 126–134.
- Edetsberger, M., Gaubitzer, E., Valic, E., Waigmann, E., & Kohler, G. (2005). Detection of nanometer-sized particles in living cells using modern fluorescence fluctuation methods. *Biochemical and Biophysical Research Communications*, 332(1), 109–116.
- Egorin, M. J., Yuan, Z. M., Sentz, D. L., Plaisance, K., & Eiseman, J. L. (1999). Plasma pharmacokinetics of butyrate after intravenous administration of sodium butyrate or oral administration of tributyrin or sodium butyrate to mice and rats. *Cancer Chemotherapy and Pharmacology*, 43(6), 445–453.
- Entin-Meer, M., Rephaeli, A., Yang, X., Nudelman, A., Nudelman, A., & Haas-Kogan, D. A. (2007). AN-113, a novel prodrug of 4-phenylbutyrate with increased anti-neoplastic activity in glioma cell lines. *Cancer Letters*, 253(2), 205–214.
- Entin-Meer, M., Rephaeli, A., Yang, X., Nudelman, A., VandenBerg, S. R., & Haas-Kogan, D. A. (2005). Butyric acid prodrugs are histone deacetylase inhibitors that show anti-neoplastic activity and radiosensitizing capacity in the treatment of malignant gliomas. *Molecular Cancer Therapeutics*, 4(12), 1952–1961.
- Farhadi, M. R., Capelle, H. H., Erber, R., Ullrich, A., & Vajkoczy, P. (2005). Combined inhibition of vascular endothelial growth factor and platelet-derived growth factor signaling: Effects on the angiogenesis, microcirculation, and growth of orthotopic malignant gliomas. *Journal of Neurosurgery*, 102(2), 363–370.
- Farokhzad, O. C., Cheng, J., Teply, B. A., Sherifi, I., Jon, S., Kantoff, P. W., et al. (2006a). Targeted nanoparticle-aptamer bioconjugates for cancer chemotherapy in vivo. *Proceedings of the National Academy of Sciences, USA*, 103(16), 6315–6320.
- Farokhzad, O. C., Karp, J. M., & Langer, R. (2006b). Nanoparticle-aptamer bioconjugates for cancer targeting. *Expert Opinion on Drug Delivery*, 3(3), 311–324.
- Ferrara, N., & Davis-Smyth, T. (1997). The biology of vascular endothelial growth factor. *Endocrine Reviews*, 18(1), 4–25.
- Ferrara, N., Gerber, H. P., & LeCouter, J. (2003). The biology of VEGF and its receptors. *Nature Medicine*, 9(6), 669–676.
- Fisher, J. L., Schwartzbaum, J. A., Wrensch, M., & Wiemels, J. L. (2007). Epidemiology of brain tumors. *Neurologic Clinics*, 25(4), 867–890, vii.
- Fishman, J. B., Rubin, J. B., Handrahan, J. V., Connor, J. R., & Fine, R. E. (1987). Receptor-mediated transcytosis of transferrin across the blood-brain barrier. *Journal of Neuroscience Research*, 18(2), 299–304.

- Fomchenko, E. I., & Holland, E. C. (2006). Mouse models of brain tumors and their applications in preclinical trials. *Clinical Cancer Research*, 12(18), 5288–5297.
- Friedman, H. S., Kerby, T., & Calvert, H. (2000). Temozolomide and treatment of malignant glioma. *Clinical Cancer Research*, 6(7), 2585–2597.
- Fukumura, D., Xu, L., Chen, Y., Gohongi, T., Seed, B., & Jain, R. K. (2001). Hypoxia and acidosis independently up-regulate vascular endothelial growth factor transcription in brain tumors in vivo. *Cancer Research*, 61(16), 6020–6024.
- Fundaro, A., Cavalli, R., Bargoni, A., Vighetto, D., Zara, G. P., & Gasco, M. R. (2000). Non-stealth and stealth solid lipid nanoparticles (SLN) carrying doxorubicin: Pharmacokinetics and tissue distribution after i.v. administration to rats. *Pharmacological Research*, 42(4), 337–343.
- Gaidamakova, E. K., Backer, M. V., & Backer, J. M. (2001). Molecular vehicle for target-mediated delivery of therapeutics and diagnostics. *Journal of Controlled Release*, 74(1–3), 341–347.
- Gasco, M. R. (2007). Lipid nanoparticles: Perspectives and challenges. *Advanced Drug Delivery Reviews*, 59(6), 377–378.
- Goppert, T. M., & Muller, R. H. (2005). Polysorbate-stabilized solid lipid nanoparticles as colloidal carriers for intravenous targeting of drugs to the brain: Comparison of plasma protein adsorption patterns. *Journal of Drug Targeting*, 13(3), 179–187.
- Gupta, Y., Jain, A., & Jain, S. K. (2007). Transferrin-conjugated solid lipid nanoparticles for enhanced delivery of quinine dihydrochloride to the brain. *The Journal of Pharmacy and Pharmacology*, 59(7), 935–940.
- Huang, G., Zhang, N., Bi, X., & Dou, M. (2008). Solid lipid nanoparticles of temozolomide: Potential reduction of cardiac and nephric toxicity. *International Journal of Pharmaceutics*, 355(1–2), 314–320.
- Jain, K. K. (2007). Use of nanoparticles for drug delivery in glioblastoma multiforme. *Expert Review of Neurotherapeutics*, 7(4), 363–372.
- Jain, R. K., di Tomaso, E., Duda, D. G., Loeffler, J. S., Sorensen, A. G., & Batchelor, T. T. (2007). Angiogenesis in brain tumours. *Nature Reviews. Neuroscience*, 8(8), 610–622.
- Jain, S. K., Chaurasiya, A., Gupta, Y., Jain, A., Dagur, P., Joshi, B., et al. (2008). Development and characterization of 5-fu bearing ferritin appended solid lipid nanoparticles for tumour targeting. *Journal of Microencapsulation*, 25(5), 289–297.
- Jansen, M., de Witt Hamer, P. C., Witmer, A. N., Troost, D., & van Noorden, C. J. (2004). Current perspectives on antiangiogenesis strategies in the treatment of malignant gliomas. *Brain Research. Brain Research Reviews*, 45(3), 143–163.
- Jiang, Z., & Sharfstein, S. T. (2008). Sodium butyrate stimulates monoclonal antibody over-expression in CHO cells by improving gene accessibility. *Biotechnology and Bioengineering*, 100(1), 189–194.
- Johansson, M., Brannstrom, T., Bergenheim, A. T., & Henriksen, R. (2002). Spatial expression of VEGF-A in human glioma. *Journal of Neuro-Oncology*, 59(1), 1–6.
- Kaur, I. P., Bhandari, R., Bhandari, S., & Kakkar, V. (2008). Potential of solid lipid nanoparticles in brain targeting. *Journal of Controlled Release*, 127(2), 97–109.
- Ke, L. D., Shi, Y. X., Im, S. A., Chen, X., & Yung, W. K. (2000). The relevance of cell proliferation, vascular endothelial growth factor, and basic fibroblast growth factor production to angiogenesis and tumorigenicity in human glioma cell lines. *Clinical Cancer Research*, 6(6), 2562–2572.
- Ke, L. D., Shi, Y. X., & Yung, W. K. (2002). VEGF(121), VEGF(165) overexpression enhances tumorigenicity in U251 MG but not in NG-1 glioma cells. *Cancer Research*, 62(6), 1854–1861.
- Kim, W. J., Yockman, J. W., Lee, M., Jeong, J. H., Kim, Y. H., & Kim, S. W. (2005). Soluble flt-1 gene delivery using PEI-g-PEG-RGD conjugate for anti-angiogenesis. *Journal of Controlled Release*, 106(1–2), 224–234.
- Koziara, J. M., Lockman, P. R., Allen, D. D., & Mumper, R. J. (2003). In situ blood-brain barrier transport of nanoparticles. *Pharmaceutical Research*, 20(11), 1772–1778.
- Koziara, J. M., Lockman, P. R., Allen, D. D., & Mumper, R. J. (2004). Paclitaxel nanoparticles for the potential treatment of brain tumors. *Journal of Controlled Release*, 99(2), 259–269.
- Koziara, J. M., Lockman, P. R., Allen, D. D., & Mumper, R. J. (2006). The blood-brain barrier and brain drug delivery. *Journal of Nanoscience and Nanotechnology*, 6(9–10), 2712–2735.
- Kreuter, J. (2001). Nanoparticulate systems for brain delivery of drugs. *Advanced Drug Delivery Reviews*, 47(1), 65–81.
- Krum, J. M., & Rosenstein, J. M. (1998). VEGF mRNA and its receptor flt-1 are expressed in reactive astrocytes following neural grafting and tumor cell implantation in the adult CNS. *Experimental Neurology*, 154(1), 57–65.
- Krutzfeldt, J., Kuwajima, S., Braich, R., Rajeev, K. G., Pena, J., Tuschl, T., et al. (2007). Specificity, duplex degradation and subcellular localization of antagomirs. *Nucleic Acids Research*, 35(9), 2885–2892.
- Lai, S. K., Hida, K., Man, S. T., Chen, C., Machamer, C., Schroer, T. A., et al. (2007). Privileged delivery of polymer nanoparticles to the perinuclear region of live cells via a non-clathrin, non-degradative pathway. *Biomaterials*, 28(18), 2876–2884.
- Lamszus, K., Ulbricht, U., Matschke, J., Brockmann, M. A., Fillbrandt, R., & Westphal, M. (2003). Levels of soluble vascular endothelial growth factor (VEGF) receptor 1 in astrocytic tumors and its relation to malignancy, vascularity, and VEGF-A. *Clinical Cancer Research*, 9(4), 1399–1405.
- le Sage, C., Nagel, R., Egan, D. A., Schrier, M., Mesman, E., Mangiola, A., et al. (2007). Regulation of the p27(Kip1) tumor suppressor by miR-221 and miR-222 promotes cancer cell proliferation. *The EMBO Journal*, 26(15), 3699–3708.
- Lee, M. J., Kim, Y. S., Kummar, S., Giaccone, G., & Trepel, J. B. (2008). Histone deacetylase inhibitors in cancer therapy. *Current Opinion in Oncology*, 20(6), 639–649.
- Lim, S. J., Lee, M. K., & Kim, C. K. (2004). Altered chemical and biological activities of all-trans retinoic acid

- incorporated in solid lipid nanoparticle powders. *Journal of Controlled Release*, 100(1), 53–61.
- Liu, X., Fortin, K., & Mourelatos, Z. (2008). MicroRNAs: Biogenesis and molecular functions. *Brain Pathology*, 18(1), 113–121.
- Lockman, P. R., Koziara, J. M., Mumper, R. J., & Allen, D. D. (2004). Nanoparticle surface charges alter blood-brain barrier integrity and permeability. *Journal of Drug Targeting*, 12(9–10), 635–641.
- Louis, D. N., Ohgaki, H., Wiestler, O. D., Cavenee, W. K., Burger, P. C., Jouvett, A., et al. (2007). The 2007 WHO classification of tumours of the central nervous system. *Acta Neuropathologica*, 114(2), 97–109.
- Louis, D. N., Pomeroy, S. L., & Cairncross, J. G. (2002). Focus on central nervous system neoplasia. *Cancer Cell*, 1(2), 125–128.
- Lu, W., Zhang, Y., Tan, Y. Z., Hu, K. L., Jiang, X. G., & Fu, S. K. (2005). Cationic albumin-conjugated pegylated nanoparticles as novel drug carrier for brain delivery. *Journal of Controlled Release*, 107(3), 428–448.
- Maier-Hauff, K., Rothe, R., Scholz, R., Gneveckow, U., Wust, P., Thiesen, B., et al. (2007). Intracranial thermotherapy using magnetic nanoparticles combined with external beam radiotherapy: Results of a feasibility study on patients with glioblastoma multiforme. *Journal of Neuro-Oncology*, 81(1), 53–60.
- Manjunath, K., & Venkateswarlu, V. (2005). Pharmacokinetics, tissue distribution and bioavailability of clozapine solid lipid nanoparticles after intravenous and intraduodenal administration. *Journal of Controlled Release*, 107(2), 215–228.
- Marcato, P. D., & Duran, N. (2008). New aspects of nanopharmaceutical delivery systems. *Journal of Nanoscience and Nanotechnology*, 8(5), 2216–2229.
- Martinez-Murillo, R., & Martinez, A. (2007). Standardization of an orthotopic mouse brain tumor model following transplantation of CT-2A astrocytoma cells. *Histology and Histopathology*, 22(12), 1309–1326.
- Martin-Villalba, A., Okuducu, A. F., & von Deimling, A. (2008). The evolution of our understanding on glioma. *Brain Pathology*, 18(3), 455–463.
- Mathieu, D., Lecomte, R., Tsanaclis, A. M., Larouche, A., & Fortin, D. (2007). Standardization and detailed characterization of the syngeneic Fischer/F98 glioma model. *The Canadian Journal of Neurological Sciences*, 34(3), 296–306.
- Mayor, S., & Pagano, R. E. (2007). Pathways of clathrin-independent endocytosis. *Nature Reviews. Molecular Cell Biology*, 8(8), 603–612.
- Mehnert, J. M., & Kelly, W. K. (2007). Histone deacetylase inhibitors: Biology and mechanism of action. *Cancer Journal*, 13(1), 23–29.
- Mehnert, W., & Mader, K. (2001). Solid lipid nanoparticles: Production, characterization and applications. *Advanced Drug Delivery Reviews*, 47(2–3), 165–196.
- Mellinghoff, I. K., Wang, M. Y., Vivanco, I., Haas-Kogan, D. A., Zhu, S., Dia, E. Q., et al. (2005). Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. *The New England Journal of Medicine*, 353(19), 2012–2024.
- Miglietta, A., Cavalli, R., Bocca, C., Gabriel, L., & Gasco, M. R. (2000). Cellular uptake and cytotoxicity of solid lipid nanospheres (SLN) incorporating doxorubicin or paclitaxel. *International Journal of Pharmaceutics*, 210(1–2), 61–67.
- Miller, A. A., Kurschel, E., Osieka, R., & Schmidt, C. G. (1987). Clinical pharmacology of sodium butyrate in patients with acute leukemia. *European Journal of Cancer & Clinical Oncology*, 23(9), 1283–1287.
- Miller, S. J. (2004). Cellular and physiological effects of short-chain fatty acids. *Mini Reviews in Medicinal Chemistry*, 4(8), 839–845.
- Minotti, G., Menna, P., Salvatorelli, E., Cairo, G., & Gianni, L. (2004). Anthracyclines: Molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. *Pharmacological Reviews*, 56(2), 185–229.
- Minow, R. A., Benjamin, R. S., Lee, E. T., & Gottlieb, J. A. (1977). Adriamycin cardiomyopathy — risk factors. *Cancer*, 39(4), 1397–1402.
- Minucci, S., & Pelicci, P. G. (2006). Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer. *Nature Reviews. Cancer*, 6(1), 38–51.
- Mischel, P. S., Shai, R., Shi, T., Horvath, S., Lu, K. V., Choe, G., et al. (2003). Identification of molecular subtypes of glioblastoma by gene expression profiling. *Oncogene*, 22(15), 2361–2373.
- Moffat, B. A., Reddy, G. R., McConville, P., Hall, D. E., Chenevert, T. L., Kopelman, R. R., et al. (2003). A novel polyacrylamide magnetic nanoparticle contrast agent for molecular imaging using MRI. *Molecular Imaging*, 2(4), 324–332.
- Muldoon, L. L., Sandor, M., Pinkston, K. E., & Neuwelt, E. A. (2005). Imaging, distribution, and toxicity of superparamagnetic iron oxide magnetic resonance nanoparticles in the rat brain and intracerebral tumor. *Neurosurgery*, 57(4), 785–796; discussion 785–796.
- Muller, R. H., & Keck, C. M. (2004). Challenges and solutions for the delivery of biotech drugs — a review of drug nanocrystal technology and lipid nanoparticles. *Journal of Biotechnology*, 113(1–3), 151–170.
- Muller, R. H., Mader, K., & Gohla, S. (2000). Solid lipid nanoparticles (SLN) for controlled drug delivery — a review of the state of the art. *European Journal of Pharmaceutics and Biopharmaceutics*, 50(1), 161–177.
- Muller, R. H., & Olbrich, C. (1999). Solid lipid nanoparticles: Phagocytic uptake, in vitro cytotoxicity and in vitro biodegradation. *Pharmazeutische Industrie*, 61, 564–569.
- Nicoloso, M. S., & Calin, G. A. (2008). MicroRNA involvement in brain tumors: From bench to bedside. *Brain Pathology*, 18(1), 122–129.
- Niola, F., Evangelisti, C., Campagnolo, L., Massalini, S., Bue, M. C., Mangiola, A., et al. (2006). A plasmid-encoded VEGF siRNA reduces glioblastoma angiogenesis and its combination with interleukin-4 blocks tumor growth in a xenograft mouse model. *Cancer Biology & Therapy*, 5(2), 174–179.
- Norden, A. D., Drappatz, J., & Wen, P. Y. (2008a). Antiangiogenic therapy in malignant gliomas. *Current Opinion in Oncology*, 20(6), 652–661.

- Norden, A. D., Drappatz, J., & Wen, P. Y. (2008b). Novel anti-angiogenic therapies for malignant gliomas. *Lancet Neurology*, 7(12), 1152–1160.
- Norden, A. D., & Wen, P. Y. (2006). Glioma therapy in adults. *Neurologist*, 12(6), 279–292.
- Nudelman, A., Gnizi, E., Katz, Y., Azulai, R., Cohen-Ohana, M., Zhuk, R., et al. (2001). Prodrugs of butyric acid. Novel derivatives possessing increased aqueous solubility and potential for treating cancer and blood diseases. *European Journal of Medicinal Chemistry*, 36(1), 63–74.
- O'Dwyer, P. J., & Catalano, R. B. (2006). Uridine diphosphate glucuronosyltransferase (UGT) 1A1 and irinotecan: Practical pharmacogenomics arrives in cancer therapy. *Journal of Clinical Oncology*, 24(28), 4534–4538.
- Olbrich, C., Bakowsky, U., Lehr, C. M., Muller, R. H., & Kneuer, C. (2001). Cationic solid-lipid nanoparticles can efficiently bind and transfect plasmid DNA. *Journal of Controlled Release*, 77(3), 345–355.
- Pardridge, W. M. (2007). Drug targeting to the brain. *Pharmaceutical Research*, 24(9), 1733–1744.
- Partlow, K. C., Lanza, G. M., & Wickline, S. A. (2008). Exploiting lipid raft transport with membrane targeted nanoparticles: A strategy for cytosolic drug delivery. *Biomaterials*, 29(23), 3367–3375.
- Parveen, S., & Sahoo, S. K. (2008). Polymeric nanoparticles for cancer therapy. *Journal of Drug Targeting*, 16(2), 108–123.
- Patnaik, A., Rowinsky, E. K., Villalona, M. A., Hammond, L. A., Britten, C. D., Siu, L. L., et al. (2002). A phase I study of pivaloyloxymethyl butyrate, a prodrug of the differentiating agent butyric acid, in patients with advanced solid malignancies. *Clinical Cancer Research*, 8(7), 2142–2148.
- Peart, M. J., Smyth, G. K., van Laar, R. K., Bowtell, D. D., Richon, V. M., Marks, P. A., et al. (2005). Identification and functional significance of genes regulated by structurally different histone deacetylase inhibitors. *Proceedings of the National Academy of Sciences, USA*, 102(10), 3697–3702.
- Peira, E., Marzola, P., Podio, V., Aime, S., Sbarbati, A., & Gasco, M. R. (2003). In vitro and in vivo study of solid lipid nanoparticles loaded with superparamagnetic iron oxide. *Journal of Drug Targeting*, 11(1), 19–24.
- Pellizzaro, C., Coradini, D., Morel, S., Ugazio, E., Gasco, M. R., & Daidone, M. G. (1999). Cholesteryl butyrate in solid lipid nanospheres as an alternative approach for butyric acid delivery. *Anticancer Research*, 19(5B), 3921–3925.
- Peoch, M., Farion, R., Hiou, A., Le Bas, J. F., Pasquier, B., & Remy, C. (2002). Immunohistochemical study of VEGF, angiopoietin 2 and their receptors in the neovascularization following microinjection of C6 glioma cells into rat brain. *Anticancer Research*, 22(4), 2147–2151.
- Petri, B., Bootz, A., Khalansky, A., Hekmatara, T., Muller, R., Uhl, R., et al. (2007). Chemotherapy of brain tumour using doxorubicin bound to surfactant-coated poly(butyl cyanoacrylate) nanoparticles: Revisiting the role of surfactants. *Journal of Controlled Release*, 117(1), 51–58.
- Podio, V., Zara, G. P., Carazzonnet, M., Cavalli, R., & Gasco, M. R. (2000a). Biodistribution of stealth and non-stealth solid lipid nanospheres after intravenous administration to rats. *The Journal of Pharmacy and Pharmacology*, 52(9), 1057–1063.
- Podio, V., Zara, G. P., Carazzonnet, M., Cavalli, R., & Gasco, M. R. (2000b). Biodistribution of stealth and non-stealth solid lipid nanospheres after intravenous administration to rats. *The Journal of Pharmacy and Pharmacology*, 52(9), 1057–1063.
- Potmesil, M. (1994). Camptothecins: From bench research to hospital wards. *Cancer Research*, 54(6), 1431–1439.
- Pouillart, P. R. (1998). Role of butyric acid and its derivatives in the treatment of colorectal cancer and hemoglobinopathies. *Life Sciences*, 63(20), 1739–1760.
- Qian, Z. M., Li, H., Sun, H., & Ho, K. (2002). Targeted drug delivery via the transferrin receptor-mediated endocytosis pathway. *Pharmacological Reviews*, 54(4), 561–587.
- Reddy, G. R., Bhojani, M. S., McConville, P., Moody, J., Mof-fat, B. A., Hall, D. E., et al. (2006). Vascular targeted nanoparticles for imaging and treatment of brain tumors. *Clinical Cancer Research*, 12(22), 6677–6686.
- Reid, T., Valone, F., Lipera, W., Irwin, D., Paroly, W., Natale, R., et al. (2004). Phase II trial of the histone deacetylase inhibitor pivaloyloxymethyl butyrate (pivanex, AN-9) in advanced non-small cell lung cancer. *Lung Cancer*, 45(3), 381–386.
- Rejman, J., Oberle, V., Zuhorn, I. S., & Hoekstra, D. (2004). Size-dependent internalization of particles via the pathways of clathrin- and caveolae-mediated endocytosis. *The Biochemical Journal*, 377(Pt. 1), 159–169.
- Rephaeli, A., Entin-Meer, M., Angel, D., Tarasenko, N., Gruss-Fischer, T., Bruachman, I., et al. (2006). The selectivity and anti-metastatic activity of oral bioavailable butyric acid prodrugs. *Investigational New Drugs*, 24(5), 383–392.
- Rich, J. N., & Bigner, D. D. (2004). Development of novel targeted therapies in the treatment of malignant glioma. *Nature Reviews. Drug Discovery*, 3(5), 430–446.
- Rong, Y., Durden, D. L., Van Meir, E. G., & Brat, D. J. (2006). 'Pseudopalisading' necrosis in glioblastoma: A familiar morphologic feature that links vascular pathology, hypoxia, and angiogenesis. *Journal of Neuropathology Experimental Neurology*, 65(6), 529–539.
- Rosenstein, J. M., & Krum, J. M. (2004a). New roles for VEGF in nervous tissue — beyond blood vessels. *Experimental Neurology*, 187(2), 246–253.
- Rosenstein, J. M., & Krum, J. M. (2004b). New roles for VEGF in nervous tissue — beyond blood vessels. *Experimental Neurology*, 187(2), 246–253.
- Rowinsky, E. K., & Donehower, R. C. (1995). Paclitaxel (taxol). *The New England Journal of Medicine*, 332(15), 1004–1014.
- Saleh, M., Stacker, S. A., & Wilks, A. F. (1996). Inhibition of growth of C6 glioma cells in vivo by expression of antisense vascular endothelial growth factor sequence. *Cancer Research*, 56(2), 393–401.
- Salomone, B., Ponti, R., Gasco, M. R., Ugazio, E., Quaglino, P., Osella-Abate, S., et al. (2000). In vitro effects of cholesteryl butyrate solid lipid nanospheres as a butyric acid pro-drug on melanoma cells: Evaluation of antiproliferative

- activity and apoptosis induction. *Clinical & Experimental Metastasis*, 18(8), 663–673.
- Sanson, M. (2008). Editorial review: Targets for glioma treatment: From bench to bedside. *Current Opinion in Oncology*, 20(6), 650–651.
- Sanson, M., Laigle-Donadey, F., & Benouaich-Amiel, A. (2006). Molecular changes in brain tumors: Prognostic and therapeutic impact. *Current Opinion in Oncology*, 18(6), 623–630.
- Santini, V., Gozzini, A., Scappini, B., Grossi, A., & Rossi Ferrini, P. (2001). Searching for the magic bullet against cancer: The butyrate saga. *Leukemia & Lymphoma*, 42(3), 275–289.
- Sathornsumetee, S., Rich, J. N., & Reardon, D. A. (2007). Diagnosis and treatment of high-grade astrocytoma. *Neurologic Clinics*, 25(4), 1111–1139, x.
- Serganova, I., Doubrovin, M., Vider, J., Ponomarev, V., Soghomonyan, S., Beresten, T., et al. (2004). Molecular imaging of temporal dynamics and spatial heterogeneity of hypoxia-inducible factor-1 signal transduction activity in tumors in living mice. *Cancer Research*, 64(17), 6101–6108.
- Serikawa, T., Kikuchi, A., Sugaya, S., Suzuki, N., Kikuchi, H., & Tanaka, K. (2006). In vitro and in vivo evaluation of novel cationic liposomes utilized for cancer gene therapy. *Journal of Controlled Release*, 113(3), 255–260.
- Serpe, L., Guido, M., Canaparo, R., Muntoni, E., Cavalli, R., Panzanelli, P., et al. (2006). Intracellular accumulation and cytotoxicity of doxorubicin with different pharmaceutical formulations in human cancer cell lines. *Journal of Nanoscience and Nanotechnology*, 6(9–10), 3062–3069.
- Serpe, L., Laurora, S., Pizzimenti, S., Ugazio, E., Ponti, R., Canaparo, R., et al. (2004). Cholesteryl butyrate solid lipid nanoparticles as a butyric acid pro-drug: Effects on cell proliferation, cell-cycle distribution and c-myc expression in human leukemic cells. *Anticancer Drugs*, 15(5), 525–536.
- Shenoy, V. S., Vijay, I. K., & Murthy, R. S. (2005). Tumour targeting: Biological factors and formulation advances in injectable lipid nanoparticles. *The Journal of Pharmacy and Pharmacology*, 57(4), 411–422.
- Shin, J. S., & Abraham, S. N. (2001). Caveolae as portals of entry for microbes. *Microbes and Infection*, 3(9), 755–761.
- Simons, K., & Ikonen, E. (1997). Functional rafts in cell membranes. *Nature*, 387(6633), 569–572.
- Singal, P. K., Li, T., Kumar, D., Danelisen, I., & Iliskovic, N. (2000). Adriamycin-induced heart failure: Mechanism and modulation. *Molecular and Cellular Biochemistry*, 207(1–2), 77–86.
- Soldati, T., & Schliwa, M. (2006). Powering membrane traffic in endocytosis and recycling. *Nature Reviews. Molecular Cell Biology*, 7(12), 897–908.
- Stan, A. C., Casares, S., Radu, D., Walter, G. F., & Brumeanu, T. D. (1999). Doxorubicin-induced cell death in highly invasive human gliomas. *Anticancer Research*, 19(2A), 941–950.
- Steiniger, S. C., Kreuter, J., Khalansky, A. S., Skidan, I. N., Bobruskin, A. I., Smirnova, Z. S., et al. (2004). Chemotherapy of glioblastoma in rats using doxorubicin-loaded nanoparticles. *International Journal of Cancer*, 109(5), 759–767.
- Stevens, P. J., Sekido, M., & Lee, R. J. (2004). A folate receptor-targeted lipid nanoparticle formulation for a lipophilic paclitaxel prodrug. *Pharmaceutical Research*, 21(12), 2153–2157.
- Stommel, J. M., Kimmelman, A. C., Ying, H., Nabioullin, R., Ponugoti, A. H., Wiedemeyer, R., et al. (2007). Coactivation of receptor tyrosine kinases affects the response of tumor cells to targeted therapies. *Science*, 318(5848), 287–290.
- Stupp, R., Mason, W. P., van den Bent, M. J., Weller, M., Fisher, B., Taphoorn, M. J., et al. (2005). Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *The New England Journal of Medicine*, 352(10), 987–996.
- Tan, C., de Noronha, R. G., Roecker, A. J., Pyrzynska, B., Khwaja, F., Zhang, Z., et al. (2005). Identification of a novel small-molecule inhibitor of the hypoxia-inducible factor 1 pathway. *Cancer Research*, 65(2), 605–612.
- Tiwari, S. B., & Amiji, M. M. (2006). A review of nanocarrier-based CNS delivery systems. *Current Drug Delivery*, 3(2), 219–232.
- Toi, M., Matsumoto, T., & Bando, H. (2001). Vascular endothelial growth factor: Its prognostic, predictive, and therapeutic implications. *The Lancet Oncology*, 2(11), 667–673.
- Tondelli, L., Ricca, A., Laus, M., Lelli, M., & Citro, G. (1998). Highly efficient cellular uptake of c-myc antisense oligonucleotides through specifically designed polymeric nanospheres. *Nucleic Acids Research*, 26(23), 5425–5431.
- Tsutsui, Y., Tomizawa, K., Nagita, M., Michiue, H., Nishiki, T., Ohmori, I., et al. (2007). Development of bionanocapsules targeting brain tumors. *Journal of Controlled Release*, 122(2), 159–164.
- Ugazio, E., Marengo, E., Pellizzaro, C., Coradini, D., Peira, E., Daidone, M. G., et al. (2001). The effect of formulation and concentration of cholesteryl butyrate solid lipid nanospheres (SLN) on NIH-H460 cell proliferation. *European Journal of Pharmaceutics and Biopharmaceutics*, 52(2), 197–202.
- Vasir, J. K., & Labhasetwar, V. (2007). Biodegradable nanoparticles for cytosolic delivery of therapeutics. *Advanced Drug Delivery Reviews*, 59(8), 718–728.
- Vijayaraghavalu, S., Raghavan, D., & Labhasetwar, V. (2007). Nanoparticles for delivery of chemotherapeutic agents to tumors. *Current Opinion in Investigational Drugs*, 8(6), 477–484.
- Visone, R., Russo, L., Pallante, P., De Martino, I., Ferraro, A., Leone, V., et al. (2007). MicroRNAs (miR)-221 and miR-222, both overexpressed in human thyroid papillary carcinomas, regulate p27Kip1 protein levels and cell cycle. *Endocrine-Related Cancer*, 14(3), 791–798.
- von Holst, H., Knochenhauer, E., Blomgren, H., Collins, V. P., Ehn, L., Lindquist, M., et al. (1990). Uptake of adriamycin in tumour and surrounding brain tissue in patients with malignant gliomas. *Acta Neurochirurgica (Wien)*, 104(1–2), 13–16.
- Vredenburgh, J. J., Desjardins, A., Herndon, J. E., II, Marcello, J., Reardon, D. A., Quinn, J. A., et al. (2007). Bevacizumab plus irinotecan in recurrent glioblastoma multiforme. *Journal of Clinical Oncology*, 25(30), 4722–4729.

- Wall, M. E., Wani, M. C., Natschke, S. M., & Nicholas, A. W. (1986). Plant antitumor agents. 22. Isolation of 11-hydroxycamptothecin from *camptotheca acuminata* decne: Total synthesis and biological activity. *Journal of Medicinal Chemistry*, 29(8), 1553–1555.
- Wan, F., You, J., Sun, Y., Zhang, X. G., Cui, F. D., Du, Y. Z., et al. (2008). Studies on PEG-modified SLNs loading vinorelbine bitartrate (I): Preparation and evaluation in vitro. *International Journal of Pharmaceutics*, 359(1–2), 104–110.
- Wang, J. X., Sun, X., & Zhang, Z. R. (2002). Enhanced brain targeting by synthesis of 3',5'-dioctanoyl-5-fluoro-2'-deoxyuridine and incorporation into solid lipid nanoparticles. *European Journal of Pharmaceutics and Biopharmaceutics*, 54(3), 285–290.
- Wang, Y. X., Hussain, S. M., & Krestin, G. P. (2001). Superparamagnetic iron oxide contrast agents: Physicochemical characteristics and applications in MR imaging. *European Radiology*, 11(11), 2319–2331.
- Williams, J., Lansdown, R., Sweitzer, R., Romanowski, M., LaBell, R., Ramaswami, R., et al. (2003). Nanoparticle drug delivery system for intravenous delivery of topoisomerase inhibitors. *Journal of Controlled Release*, 91(1–2), 167–172.
- Wissing, S. A., Kayser, O., & Muller, R. H. (2004). Solid lipid nanoparticles for parenteral drug delivery. *Advanced Drug Delivery Reviews*, 56(9), 1257–1272.
- Wong, H. L., Bendayan, R., Rauth, A. M., Li, Y., & Wu, X. Y. (2007). Chemotherapy with anticancer drugs encapsulated in solid lipid nanoparticles. *Advanced Drug Delivery Reviews*, 59(6), 491–504.
- Wong, H. L., Bendayan, R., Rauth, A. M., & Wu, X. Y. (2004). Development of solid lipid nanoparticles containing ionically complexed chemotherapeutic drugs and chemosensitizers. *Journal of Pharmaceutical Sciences*, 93(8), 1993–2008.
- Wong, H. L., Bendayan, R., Rauth, A. M., Xue, H. Y., Babakhanian, K., & Wu, X. Y. (2006a). A mechanistic study of enhanced doxorubicin uptake and retention in multidrug resistant breast cancer cells using a polymer-lipid hybrid nanoparticle system. *The Journal of Pharmacology and Experimental Therapeutics*, 317(3), 1372–1381.
- Wong, H. L., Rauth, A. M., Bendayan, R., Manias, J. L., Ramaswamy, M., Liu, Z., et al. (2006b). A new polymer-lipid hybrid nanoparticle system increases cytotoxicity of doxorubicin against multidrug-resistant human breast cancer cells. *Pharmaceutical Research*, 23(7), 1574–1585.
- Xu, H., Lv, M., & Tian, X. (2009). A review on hemisynthesis, biosynthesis, biological activities, mode of action, and structure-activity relationship of podophyllotoxins: 2003–2007. *Current Medicinal Chemistry*, 16(3), 327–349.
- Xu, Z., Gu, W., Huang, J., Sui, H., Zhou, Z., Yang, Y., et al. (2005). In vitro and in vivo evaluation of actively targetable nanoparticles for paclitaxel delivery. *International Journal of Pharmaceutics*, 288(2), 361–368.
- Yang, S. C., Lu, L. F., Cai, Y., Zhu, J. B., Liang, B. W., & Yang, C. Z. (1999). Body distribution in mice of intravenously injected camptothecin solid lipid nanoparticles and targeting effect on brain. *Journal of Controlled Release*, 59(3), 299–307.
- Yang, W., Barth, R. F., Wu, G., Kawabata, S., Sfera, T. J., Bandyopadhyaya, A. K., et al. (2006a). Molecular targeting and treatment of EGFRvIII-positive gliomas using boronated monoclonal antibody L8A4. *Clinical Cancer Research*, 12(12), 3792–3802.
- Yang, W., Barth, R. F., Wu, G., Kawabata, S., Sfera, T. J., Bandyopadhyaya, A. K., et al. (2006b). Molecular targeting and treatment of EGFRvIII-positive gliomas using boronated monoclonal antibody L8A4. *Clinical Cancer Research*, 12(12), 3792–3802.
- Yang, W., Wu, G., Barth, R. F., Swindall, M. R., Bandyopadhyaya, A. K., Tjarks, W., et al. (2008). Molecular targeting and treatment of composite EGFR and EGFRvIII-positive gliomas using boronated monoclonal antibodies. *Clinical Cancer Research*, 14(3), 883–891.
- Yano, A., Shingo, T., Takeuchi, A., Yasuhara, T., Kobayashi, K., Takahashi, K., et al. (2005). Encapsulated vascular endothelial growth factor-secreting cell grafts have neuroprotective and angiogenic effects on focal cerebral ischemia. *Journal of Neurosurgery*, 103(1), 104–114.
- Yasuhara, T., Shingo, T., Muraoka, K., wen Ji, Y., Kameda, M., Takeuchi, A., et al. (2005). The differences between high and low-dose administration of VEGF to dopaminergic neurons of in vitro and in vivo parkinson's disease model. *Brain Research*, 1038(1), 1–10.
- Zara, G. P., Bargoni, A., Cavalli, R., Fundaro, A., Vighetto, D., & Gasco, M. R. (2002a). Pharmacokinetics and tissue distribution of idarubicin-loaded solid lipid nanoparticles after duodenal administration to rats. *Journal of Pharmaceutical Sciences*, 91(5), 1324–1333.
- Zara, G. P., Cavalli, R., Bargoni, A., Fundaro, A., Vighetto, D., & Gasco, M. R. (2002b). Intravenous administration to rabbits of non-stealth and stealth doxorubicin-loaded solid lipid nanoparticles at increasing concentrations of stealth agent: Pharmacokinetics and distribution of doxorubicin in brain and other tissues. *Journal of Drug Targeting*, 10(4), 327–335.
- Zara, G. P., Cavalli, R., Fundaro, A., Bargoni, A., Caputo, O., & Gasco, M. R. (1999). Pharmacokinetics of doxorubicin incorporated in solid lipid nanospheres (SLN). *Pharmacological Research*, 40(3), 281–286.
- Zhang, Y., Jeong Lee, H., Boado, R. J., & Pardridge, W. M. (2002). Receptor-mediated delivery of an antisense gene to human brain cancer cells. *The Journal of Gene Medicine*, 4(2), 183–194.
- Zhang, Y., Zhang, Y. F., Bryant, J., Charles, A., Boado, R. J., & Pardridge, W. M. (2004). Intravenous RNA interference gene therapy targeting the human epidermal growth factor receptor prolongs survival in intracranial brain cancer. *Clinical Cancer Research*, 10(11), 3667–3677.
- Zhou, Y. H., Tan, F., Hess, K. R., & Yung, W. K. (2003). The expression of PAX6, PTEN, vascular endothelial growth factor, and epidermal growth factor receptor in gliomas: Relationship to tumor grade and survival. *Clinical Cancer Research*, 9(9), 3369–3375.