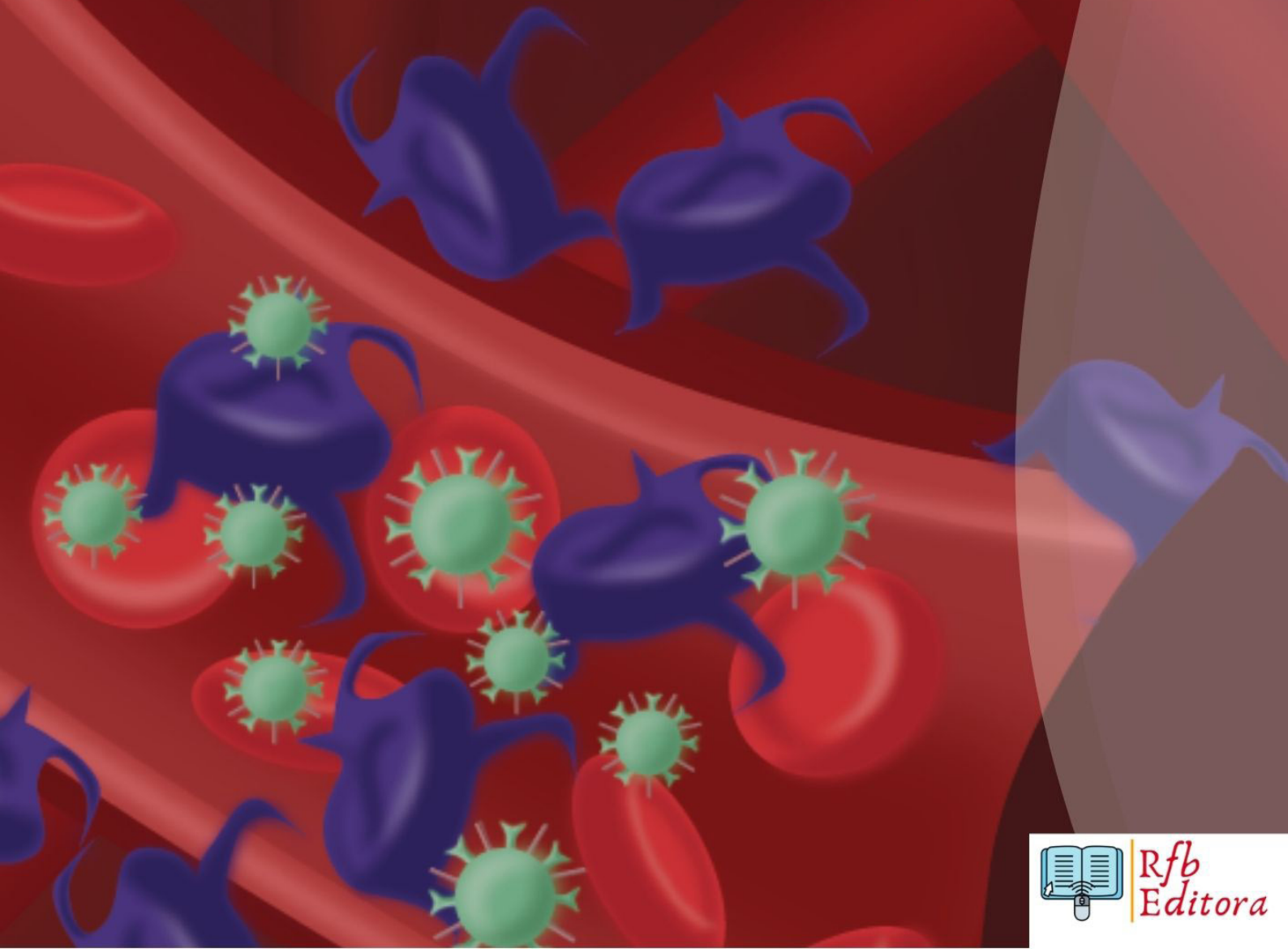
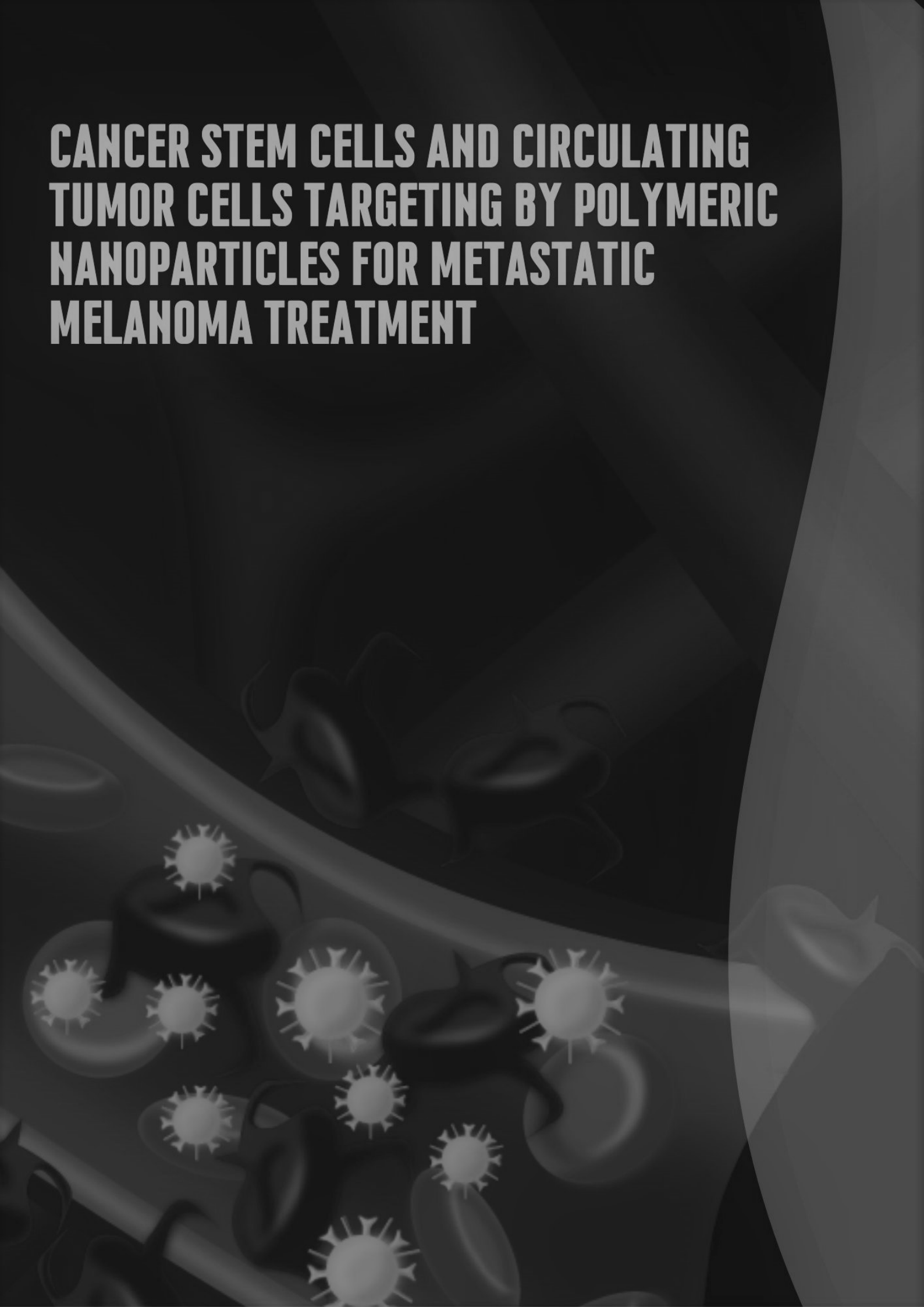
CANCER STEM CELLS AND CIRCULATING TUMOR CELLS TARGETING BY POLYMERIC NANOPARTICLES FOR METASTATIC MELANOMA TREATMENT

Sarah Brandão Palácio (Org.)
An Young Sarahi Taylor Castillo
Francisco Humberto Xavier Junior
Isabella Macário Ferro Cavalcanti (Org.)



Rfb Editora

CANCER STEM CELLS AND CIRCULATING TUMOR CELLS TARGETING BY POLYMERIC NANOPARTICLES FOR METASTATIC MELANOMA TREATMENT



Sarah Brandão Palácio (Org.)
An Young Sarahi Taylor Castillo
Francisco Humberto Xavier Junior
Isabella Macário Ferro Cavalcanti (Org.)

CANCER STEM CELLS AND CIRCULATING TUMOR CELLS TARGETING BY POLYMERIC NANOPARTICLES FOR METASTATIC MELANOMA TREATMENT

Edição 1

Belém-PA

Rfb Editora

2021

https://doi.org/10.46898/rfbe.9786558890249

Dados Internacionais de Catalogação na Publicação (CIP).

R159

Cancer STEM cells and circulating tumor cells targeting by polymeric nanoparticles for metastatic melanoma treatment [recurso digital] / Sarah Brandão Palácio (Org.), An Young Sarahi Taylor Castillo, Francisco Humberto Xavier Junior, Isabella Macário Ferro Cavalcanti (Org.) -- 1. ed. -- Belém: RFB Editora, 2021.
4.648 kB; PDF: il.
Inclui Bibliografia.
Modo de acesso: World Wide Web.

ISBN: 978-65-5889-024-9
DOI: 10.46898/rfbe.9786558890249

1. Tumores e câncer. 2. Pesquisa. 3. Estudo. I. Título.

CDD 616.99

Rfb Editora

Nossa missão é a difusão do conhecimento gerado no âmbito acadêmico por meio da organização e da publicação de livros digitais de fácil acesso, de baixo custo financeiro e de alta qualidade!

Nossa inspiração é acreditar que a ampla divulgação do conhecimento científico pode mudar para melhor o mundo em que vivemos!

Equipe RFB Editora

Diagramação:

Danilo Wothon Pereira da Silva.

Design da capa:

Taylane Britto

Revisão de texto:

Os autores.

Home Page: www.rfbeditora.com.
E-mail: adm@rfbeditora.com.
Telefone: (91)3085-8403/98885-7730.
CNPJ: 39.242.488/0001-07.
Barão de Igarapé Miri, sn, 66075-971, Belém-PA.

SUMMARY

ACKNOWLEDGMENTS

The authors thank the international financial support of Coordination for the Improvement of Higher Education Personnel (CAPES) and French Committee for the Evaluation of Academic and Scientific Cooperation with Brazil (COFECUB) (grant #721/11). Sarah Brandão Palácio also thanks the for co-tutele PhD scholarship (COFECUB nº 4696/14-0).

PRESENTATION

The nanotechnology has been widely applied in the diagnosis and treatment of several diseases. Nanotechnology applications in cancer based on drug delivery systems have been extensively evaluated over last decade and demonstrated to be a promising approach to improve the efficacy of anti-cancer therapy. However, the cellular heterogeneity and plasticity presented in tumors sites represent one of the main causes of metastasis and can be considered as one of the most challenge subjects for the improvement of cancer therapeutics. In this context, one of the cancer types with greatest metastatic potential and chemotherapeutic resistance is the melanoma. Precisely, two types of cancer cells are directly involved in tumor heterogeneity and melanoma metastasis: the cancer stem cells (CSCs) and circulating tumor cells (CTCs). High levels of CSCs and CTCs has been associated with tumor progression, chemoresistence and metastatic spread.

Owing to the clinical relevance of this skin cancer, the melanoma treatment has been widely explored for nanotechnology applications, including the use of polymeric nanoparticles. This book was dedicated to present and discuss the status of melanoma biomarkers and to evaluate the advances in polymeric nanoparticles strategies in order to develop effective drug delivery systems for the treatment of metastatic melanoma.

CHAPTER 1

INTRODUCTION

Melanoma is a skin cancer characterized by the malignant transformations of melanocytes of neural crest origin, different from non-melanoma skin cancers, which are originating from the basal and squamous cell layers in the epidermis. Melanoma is the most aggressive skin cancer and exhibits resistance to current treatments (Cichorek *et al*., 2013; Slominski & Carlson, 2015). Although melanoma accounts for only 1% of all skin cancers, it is the major cause of poor prognostics and deaths due to its markedly metastatic potential. Extensive research efforts have been made over the past years to better understand the melanoma metastasis mechanisms/pathogenesis and which factors, environmental and genetics, are involved in disease progression (Landow *et al*., 2016; Pietila *et al*., 2016).

In general, different types of cancer cells are found within the same tumor site and these phenotypic and functional heterogeneity can be a result of an extensive genetic and epigenetic instability, cell plasticity and tumor microenvironmental characteristics (Meacham & Morrison, 2013; Sun & Yu, 2015). These intratumor heterogeneity are closely related to metastatic disease and represents the main cause of anti-cancer therapy resistance, therefore it is a challenging subject for the improvement of cancer patient's survival (Brooks *et al*., 2015; Gay *et al*., 2016). Two main concepts have been proposed to explain the intratumor heterogeneity and cancer progression: stochastic model and cancer stem cells (CSCs) model. The stochastic or clonal evolution preconizes that differences between tumorigenic cancer cells are generated through genetic and epigenetic mechanisms and lack hierarchical organization. On the other hand, the CSC model hypothesizes that cancer is hierarchically organized into nontumorigenic and tumorigenic fractions. These mesenchymal cancer cells with tumorigenic potential represents the minority population in tumor environment and has capacity of self-renewing and generate heritable phenotypic variation (Csermely *et al*., 2014; Brooks *et al*., 2015). The pluripotent characteristics of the CSCs have been considered the driving force of tumor progression and these cells are involved in metastatic dissemination due to their ability to initiate and sustain the cancer disease (La Porta & Zapperi, 2013). Studies suggested the existence of these tumor cells subpopulation in *in vivo* melanoma models (Dou *et al*., 2007; Sigalotti *et al*., 2008; Shakhova & Sommer, 2013).

Another type of cancer cells, directly related to tumor heterogeneity and involved in melanoma metastasis development, are the circulating tumor cells (CTCs). This cancer cells are shed in peripheral blood from a primary or metastatic tumor (Xu & Zhong, 2010). Epithelial CTCs were first reported over 100 years ago (Ashworth, 1869) and since then, the isolation and identification of these cells by specific biomarkers displayed at their surface or intracellularly are used to evaluate cancer prognostics, have been correlated with metastatic disease and poor patient outcome (Ashworth, 1869; Lianidou *et al*., 2015).

In recent years, the biological understanding of invasive and metastatic capability of CSCs and CTCs have attracted increasing attention in target these cells for diagnosis, prognostics and clinical treatment of melanoma cancer. Nowadays, besides the prognostics properties, the identification of CSCs and CTCs biomarkers have been described as a potent clinical approach to optimize the chemotherapeutic scheme and to develop personalized targeted systems for cancer therapy, improving the survival in patients with a variety of solid tumors, such as melanoma (La Porta & Zapperi, 2013; Li *et al*., 2015; Zhang *et al*., 2016).

The conventional anti-cancer monotherapy based on systemic administration of cytotoxic agents, such as paclitaxel, cisplatin and doxorubicin, are commonly ineffective in metastatic diseases, presenting poor pharmacokinetics properties, dose-limiting toxicities and induction of drug resistant cancer cells (Grundy *et al*., 2016). Recent studies have been suggested that combination therapies of cytotoxic agents, as dacarbazine, with newer molecularly targeted inhibitors, as vemurafenib and trametinib, or immunotherapy agents, as ipilimumab, are the most promising strategy to achieving long-term sustained response, decrease the relapse rate and increase the overall survival rate for patients with metastatic melanoma (Davey *et al*., 2016; Tran *et al*., 2016). Despite of the increasing effort in propose new therapeutic schemas for metastatic melanoma, these treatments have limited effectiveness and serious health-threatening effects (Eroglu & Ribas, 2016).

In last decade, the nanotechnology approach, based on the versatile and modifiable drug delivery nanocarriers systems, as polymeric nanoparticles, has been an extensively explored strategy to overcome the hazards related to conventional anti-cancer therapy (Drewes *et al*., 2016; Silva *et al*., 2016). Nanotechnology applications based on passive and active targeted drug delivery systems demonstrated to be a very promising technology to improve the efficacy of melanoma diagnostic and therapy (Bombelli *et al*., 2014; Silva *et al*., 2015; Kumari & Kondapi, 2016). The passive targeting is based on the nonspecific accumulation of nanocarriers on tumor microenvironment by the enhanced permeability and retention effect (EPR effect), whereas the active targeting is based on the affinity of target moieties attached at nanocarrier surface to specifically recognize biomarkers on the tumor cells (Upponi *et al*., 2014). Researchers are being carried out using polymeric nanoparticles in order to enhance the transport of active or/and imaging agents across biological barriers and also to recognize specific tumor markers at the surface of melanoma cells (Drewes *et al*., 2016). Nevertheless, the use of nanotechnology to target CSCs and CTCs, in order to impair tumor progression and prevent metastasis, is still emerging and being purposed in current research (Li *et al*., 2015; Garcia-Mazas *et al*., 2016). The intrinsic physicochemical characteristics of polymeric nanoparticles, such as the large surface area, modifiable surface properties and

long circulation half-life, represent a promising path to development of drug delivery systems to reach the heterogeneous cancer cell population, including the CSCs and CTCs that have a high metastatic potential and are not easily targeted (Li *et al*., 2015; Zuo *et al*., 2016).

In these perspectives, the first part of this book provided an overview of the current research regarding biological mechanisms of CSCs and CTCs in metastatic development and their related biomarkers detected in melanoma patients. In the second part, the recent advances in polymeric nanoparticles for passive and active targeting to advanced melanoma treatment are presented and discussed. This section focused on CSCs and CTCs targeting strategies and on the influence of architectural properties of nanoparticles in their *in vivo* performance. In summary, this book aims to shed light on the potential biomarkers for nanotechnology applications, specially in polymeric nanoparticles, to target CSCs and CTCs and to improve metastatic melanoma treatment.

CHAPTER 2

CANCER STEM CELLS (CSCS) AND CIRCULATING TUMOR CELLS (CTCS) ROLE IN MELANOMA TUMOR

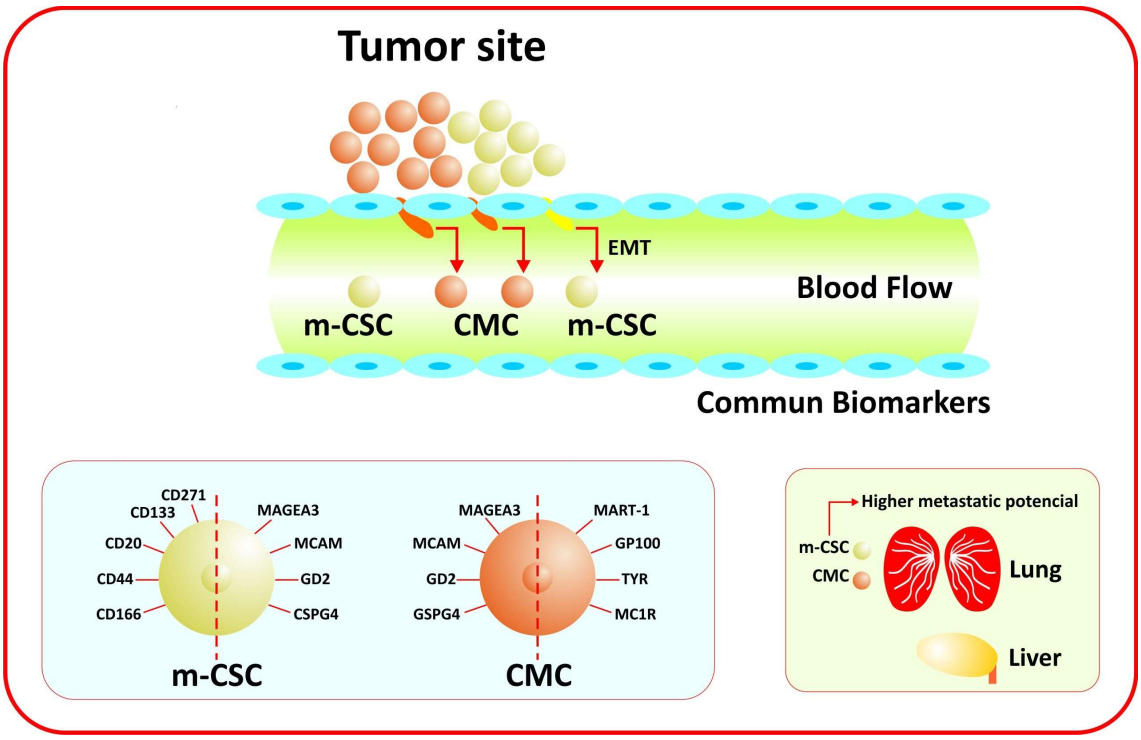
CSCS

Since Briggs & King (1952) challenged the unidirectional development model of embryonic stem cells that differentiates into somatic cells, several studies also demonstrated that normal cells and tumor cells could go back in time through the dedifferentiation process. This process is defined as the reversion of cells from a differentiated state to an embryonic state with less specialized characteristics and functions (Jopling *et al*., 2011).

The dedifferentiation process and genetic mutation of normal stem/progenitor cells, somatic cells and cancer cells, leading by pro-oncogenes, may result in a transformation into a self-renewing and multipotent type of tumorigenic cells called cancer stem cells (CSCs) (Borovski *et al*., 2011; Friedmann-Morvinski & Verma, 2014). The CSCs have been demonstrated to play an important role in tumor progression, including melanoma (Herreros-Villanueva *et al*., 2013; Shakhova & Sommer, 2013). These cells are considered to be a rare subpopulation of tumor niche and they have a long--term proliferative ability and capacity for asymmetrical division, besides are involved in angiogenic induction and apoptotic resistance, including resistance to chemo-radiation therapy. Numerous researches appointed that the CSCs characteristics are intimately involved in metastasis development and cancer relapse (Shiozawa *et al*., 2013; Allegra *et al*., 2014). Thanks to these features, the CSCs becomes a new therapeutic target for cancer treatment, specially for tumor progression impairment, as well as, a valuable biological marker for cancer prognostics, since they could be detected in majority of malignant tumors (Vinogradov & Wei, 2012; Bao *et al*., 2013).

The mechanisms that may originate the CSCs are not fully understood yet. One of the key mechanism for generation of CSC phenotype cells in tumor site is the epidermal to mesenchymal transition (EMT) (Figure 1). Briefly, this mechanism consists in an activation of the embryogenic state of cancer cells located at the primary tumor (Borovski *et al*., 2011). Studies demonstrated that cell fusion, horizontal gene transfer and microenvironment conditions such as hypoxia and induction factors as transforming growth factor-β (TGF-β), could promote CSC formation, proliferation and clonal selection of CSCs (Lobo *et al*., 2007; Borovski *et al*., 2011).

Figure 1 - Schematic illustration of tumor site derived circulating melanoma cells (CMC) and melanoma cancer stem cells (m-CSCs) in blood flow and representation of the epidermal to mesenchymal transition (EMT) processes. The CMC and m-CSCs possess high metastatic potential and disseminated preferentially to the lung and liver. The main known biomarkers for m-CSCs and CMC are shown in scheme below, highlighting the common markers.



Source: Authors.

Owing to the concept that tumors are characterized by their cell heterogeneity, CSCs can be considered a side cell population of tumor that have unlimited proliferation capability, potential to differentiate, accumulate genetic mutations and consequently may presented a strong tumorigenicity, giving rise to various population of tumor cells. Nevertheless, depending on the type of cancer, CSCs concept was not completely accepted yet, specially due to the controversial results of current research that identify these cells in tumor sites (Kelly *et al*., 2007; Visvader & Lindeman, 2012; Shiozawa *et al*., 2013).

The evidences suggest a controversy regarding the existence of CSCs in tumor microenvironment. Bonnet & Dick (1997) demonstrated one of the first evidence of CSCs hypothesis thought the acute myeloid leukemia model. In this research was observed that the leukemogenic event was originate by primitive cells that expressed the specific markers on their surface, as the $CD34^+$ and $CD38^-$, and had the capability to prevent the normal differentiation occurrence. Challenging the CSCs concept, Kelly and colleges (2007) injected mouse lymphoma cells into nonirradiated congenic animals and demonstrated that all animals developed fatal lymphoma, suggesting that the CSCs were not the only tumor initiating cells. In the same way, Quintana and collegues (2010) investigated the tumorigenic capacity of melanoma cells. This study demonstrated that cancer cells with different biomarkers and phenotypes had the

potential to form tumors and that melanoma exhibited a phenotypic plasticity, which contrasts with CSCs model that hypothesizes irreversible genetic changes by tumorigenic cells.

Despite these controversial studies, most of the evidences indicate the existence of CSCs in melanoma tumor models and indicate that these cells can constitute a small fraction (0.0001 to 1%) of heterogeneous cells population in tumor microenvironment (Dou *et al*., 2007; Schatton *et al*., 2008). Meanwhile, other researchers suggest that the frequency of tumorigenic cells in primary tumors could be underestimated by the xenograft transplantation model, the most applied *in vivo* model to demonstrate the CSCs existence (Kelly *et al*., 2007; Zhong *et al*., 2010; Jandl et al., 2013). Furthermore, Kelly and collegues (2007) hypothesize that the presence and frequency of CSCs strongly depend of the tumor type, specially due to the variable degrees of functional heterogeneity as consequence of the specific oncogenic pathways. Researches have been proposed new methods to improve the detectable frequency of CSCs applying more severely immunocompromised mice. Quintana and coworkers (2008) modified xenotransplantation assay conditions, using non-obese diabetic combined immunodeficiency (NOD/SCID) interleukin-2 receptor gamma chain null (Il2rg2/2) mice and observed a dramatically increase, by several orders of magnitude, of detectable melanoma tumorigenic cells. Similarly, Zhong and coworkers (2010) also found a substantial population of CSCs (> 10%) in B16-F10 melanoma cells in syngeneic mice.

In view of to shed light on definition, origin, identification and frequency of CSCs *in vivo*, it is essential to identify the expression of appropriate surface markers, which could be able to distinguish tumorigenic melanoma CSCs by their distinct functions from the normal tumor cells that are non-tumorigenic.

CTCS

The 'seed and soil theory' proposed by Paget (1889) preconizes that the metastasis' formation is a nonrandom process where the CTCs (seeds) target specific organs that presents a desirable microenvironment (soil) for tumor cells growth. It is already well established that CTCs are cells located in peripheral blood directly involved in the spread of tumor cells from an organ-confined site to distant sites, resulting in metastases to multiple organs. However, the transition of cancer cells, derivate from primary tumors, to blood circulation can be very drastic for the cells and they need to acquire a special phenotype to survive to the harsh conditions of an anchorage-independent environment. One of the most critical mechanisms involved in CTCs production is epidermal to mesenchymal transition (EMT). This epithelial cancer cell transition to mesenchymal state helps to maintain the invasive phenotype and metastatic potential of CTCs (Borovski *et al*., 2011). Growing evidences indicates that cancer cells loss their

cell-cell junctions after EMT, becoming more motile and aggressive, allowing more efficient cancer cell metastasis (Zhang *et al*., 2016). EMT process occurs specially as a result of combined epigenetic mutations and changes in tumor microenvironment and directly influences the regulation of tumor development (Pietila *et al*., 2016).

CTCs are part of tumor cellular heterogeneity and the mainly difficult in obtain a suitable molecular definition for these cells is their rarity in blood. It is estimated to exist 1 among 10^6 to 10^7 normal white blood cells (Ross *et al*., 1993) or 1 to 10 CTCs for each 4 mL of blood of metastatic melanoma patients (Freeman *et al*., 2012). A recent mini-review exalted the importance of CTCs detection and characterization in the blood of cancerous patients as an alternative to invasive tissue biopsies and to improve the cancer prognostics (Zhang *et al*., 2016). This new prognostic strategy has been called liquid biopsy and could be very helpful to elucidate how the CTCs gain resistance against anti-cancer treatments. Therefore, CTCs isolated from blood flow through non-invasive method could allow various clinical advantages: monitoring phenotypic changes in cancer cells of metastatic patients; detection of this markers as an indicative of tumor progression; discovered future targets to individualized cancer therapies, apart lead to a better understand of cancer cell biology and metastasis mechanisms (Lianidou *et al*., 2015). The available methods to CTCs isolation and detection in blood flow can include antibody-based capture assays, size-based filtration or nucleic acid-based assays (Pore *et al*., 2016). Unluckily, these methods are very technically limited, specially due to the sparse number of CTCs in circulating blood of cancer patients (Adams *et al*., 2015). Xu & Zhong (2010) reinforce the necessity to discovery miniaturized methods, specially using the nanotechnology, that allows fully characterized CTCs in a single-cell level. Due to this expressively role of CTCs in metastases pathogenesis, these cells have become a very promising target to evaluate the patient's prognostics and to develop new treatment strategies to prevent cancer dissemination (Hayes & Paoletti, 2013).

Nevertheless, it has been reported that individual CTCs are not the only responsible for metastatic progression. In addition, aggregates of CTCs, referred to CTCs clusters or circulating tumor microemboli, demonstrated to play a key role in tumor dissemination (Hai *et al*., 2017; Au *et al*., 2017). These clusters of tumor cells were discovered in the 1950's, however due to the lack of suitable techniques for CTCs clusters isolation for the past decades, the ability of these cell aggregates to generate metastasis were only evidenced recently (Hong *et al*., 2016). Studies showed that the CTCs clusters, despite to be in inferior amounts than single CTCs in blood flow, have a higher metastatic potential, ranging from about 25 to 100 folds, when compared to individual CTCs (Lione te al., 1978; Aceto *et al*., 2014; Au *et al*., 2016).

In general, CTCs clusters have a heterogeneous composition, including ephitelial and mesenchymal tumor cells, non-tumor cells, macrophages, endothelial and epithelial cells, fibroblasts and platelets. This tumor derived stromal cells are responsible to impair the immune attack of CTCs clusters and increase the viability of tumor cells, specially protecting them from the shear forces in the blood stream and enhancing the tumor progression (Aceto *et al*., 2014; Hong *et al*., 2016; Khoo *et al*., 2017).

The isolation and characterization of CTCs clusters can offer important new insights for the development of more effective anticancer therapies and better elucidate the metastastic process (Khoo *et al*., 2017). Among the techniques for isolation of CTCs clusters, antibody-based methods are not very effective due to the higher surface area of CTC clusters compared to single CTCs, which hinders the antibody capture (Hong *et al*., 2016). The most promising methods developed recently for CTCs clusters detection and isolation are based on the physical properties of these cell groups, such as the size, combined with microfluidic technology (Khoo *et al*., 2017; Chiu *et al*., 2018). Despite of the more critical role of CTCs clusters in metastasis compared to individual CTCs, little is known about the mechanisms of metastasis development by CTCs groups and how the tumor cell clusters microenvironment supports their viability in blood flow. In this way, it is very important to develop new plataforms for detection of intact CTCs clusters and make feasible their clinical application.

CHAPTER 3

CLINICAL RELEVANCE OF MELANOMA CANCER STEM CELLS AND CIRCULATING MELANOMA CELLS

Biomarkers are molecules that can be measured and evaluated as indicative of normal biological processes or pathological conditions, such as cancer. There is a wide variety of biomarkers including transmembrane proteins (e.g., receptors), glycoproteins (e.g., integrins), nucleic acids (e.g., microRNAs), transcription factors, carbohydrates, hormones, and antibodies (Schwarzenbach *et al*., 2011; Sethi *et al*., 2013). In recent years, significant efforts have been made to better characterize biomarkers in oncology, which play a critical role in initiation, progression, and maintenance of tumors. In melanoma skin cancers, a large number of biomarkers, mainly proteins, have been identified in melanoma cells and their expression have been correlated with different stages of melanocytic tumor progression (Marconi *et al*., 2015). In this context, the identification and recognition of biomarkers expressed in two particular types of metastatic melanoma cells, melanoma cancer stem cells (m-CSCs) and circulating melanoma cells (CMCs), have been reported as an important strategy to provide an accurate diagnosis and improve the therapy of this skin cancer (Freeman *et al*., 2012; Schlaak *et al*., 2012; La Porta & Zapperi, 2013).

The biomarkers can be non-invasively assessed and detected in body fluids as blood, urine, feces and sputum, or invasively assessed by a tissue biopsy (Henry & Hayes, 2012; Xiao *et al*., 2013). High throughput technologies have been adopted to identify and characterize potential biomarkers include positron emission tomography, protein microarray, exome sequencing, flow/mass cytometry, multicolor immunohistochemistry and capillary electrophoresis (Sethi *et al*., 2013; Yuan *et al*., 2016).

The detection and characterization of m-CSCs and CMCs by accurate techniques could allow to clinicians establishes more effective prognostics and infers the melanoma metastatic risk of current patients as well as the relapse disease potential of patients that were submitted by curative resections (Huang & Hoon, 2016). Despite the clinical relevance of these cells, the most challenge drawbacks to clinical applications of m-CSCs and CMCs is their heterogeneity of biomarker expression and the isolation of these cells from tumor sites and blood circulation (La Porta & Zapperi, 2013; Gray *et al*., 2015; Zand *et al*., 2016).

In the next two chapters of this book, we describe the main biomarkers reported in literature for m-CSC and CMCs (Figure 1 and Table 1), their role in metastasis and their current clinical applications in diagnosis and treatment of melanoma.

Table 1 - Current biomarkers detected in m-CSC and CMC.

Biomarkers	Type	Cellular localization	Main functions/ Mechanisms	References
(a) Mesenchymal cancer stem cells (m-CSC)				
CD133	TM/IgSF	Plasma Membrane	Signaling function/ cell differentiation into tumor endothelium	Mak *et al.*, 2014 Borovski *et al.*, 2011 Wu *et al.*, 2009
CD271	TM/IgSF/ TNFR	Plasma Membrane	Cell survival, apoptosis and adhesion/ regulation of β1-integrin	Valyi-Nagy *et al.*, 2012
CD166	TM/IgSF/ Alcam	Plasma membrane	Cell growth, migration and adhesion/ heterophilic and homophilic cell-cell interactions	Weidle *et al.*, 2014 Swart, 2002
CD44	TM/IgSF/ HA-R	Plasma membrane	Cell adhesion, migration/ heterophilic and homophilic cell-cell interactions	Ahrens *et al.*, 2001 Faaseen *et al.*, 1992
CD20	TM/IgSF/B-cell-specific cell-surface molecule	Plasma Membrane	Regulates cell-cycle progression of B lymphocytes/ Ca^{+2} channel activity	Cragg *et al.*, 2004 Tedder & Engel,1994
(b) Circulating Melanoma cells (CMC)				
MART-1	Protein/ MDA	Cytosol (Golgi complex, ER and melanosomes), plasma membrane (HLA- restricted epitope)	Melanocyte differentiation, biosynthesis of melanin and T-cells recognition	Ordóñez, 2013 Mazière *et al.*, 2002 Rimoldi *et al.*, 2001
GP100	Protein/ MDA	Cytosol (melanosomes), plasma membrane (HLA-restricted epitope)	Melanocyte differentiation, biosynthesis of melanin and T-cells recognition	Ordóñez, 2013 Mazière *et al.*, 2002
TYR	Protein/ MDA	Cytosol (melanosomes), plasma membrane (HLA-restricted epitope)	Melanocyte differentiation, biosynthesis of melanin and T-cells recognition	Ordóñez, 2013 Mazière *et al.*, 2002
MC1R	T-GPR	Cytosol, plasma Membrane	Regulates the production of melanin (eumelanin and pheomelanin) by melanocytes	Rees, 2000 López *et al.*, 2007
(c) Common Markers (m-CSC and CMC)				
MAGEA3	Protein/ MDA	Cytosol, plasma membrane (HLA-restricted epitope)	Cell cycle progression and apoptosis/ Immune response against cancer	Sigalotti *et al.*, 2002b Rimoldi *et al.*, 2001
MCAM/ CD146	TM/IgSF	Plasma Membrane	Support endothelial integrity, lymphocyte recruitment/ Cell adhesion, migration, homing, and inflammation /homotypic and heterotypic cell interactions	Duan *et al.*, 2013 Ouhtit *et al.*, 2009 Elshal *et al.*, 2007

GD2	Ganglioside	Plasma Membrane	Cell adhesion/invasion and/or proliferation/ modulate intracellular and intranuclear calcium homeostasis	Yu *et al.*, 2011 Horta *et al.*, 2016
CSPG4	T-PG	Plasma Membrane	Cell adhesion, growth, motility and survival; angiogenesis/ activation of integrins and GTPase family proteins	Wang *et al.*, 2010 Wang *et al.*, 2011b Price *et al.*, 2011

BIOMARKERS FOR M-CSCS

A novel and promising clinical approach to improve the prognostics of patients with metastatic melanoma is the targeting of m-CSCs, specially through immunotherapy in both tumor site and blood flow. Unfortunately, despite of the increase research efforts to understand the antigenic profile of these cells, little is known about the expression of specific tumor-associated antigens and which are the triggers and microenvironmental conditions that regulates these antigens expressions in m-CSCs (Sigalotti *et al.*, 2008). Nevertheless, it is well-known that normal and tumor pluripotent cells generally displays cluster differentiation markers at the cell surface and that m-CSCs also overexpressed specific cluster differentiation antigens, such as CD133, CD44, CD20 and CD271 (Shmelkov *et al.*, 2008; Singh & Settleman, 2010; Morath *et al.*, 2016).

In this context, CD133 is one of the most well characterized antigen presented in the surface of normal stem cells and CSCs derivate from a wide range of tissue types and its epitope AC133 has also been highly used as an important biomarker to detected and isolated CSCs (Mak *et al.*, 2014). The CD133 is a 120-kDa transmembrane glycoprotein, expressed in plasma membrane and their expression have been associated with chemoresistance and radioresistance in various cancer types (Ferrandina *et al.*, 2009; Wu & Wu, 2009). Recent evidences support that CD133 plays an essential role in establishing the vascular niche through the cell differentiation into tumor endothelium, however the mechanisms that govern its function require further elucidation (Mak *et al.*, 2014). CSCs expressing CD133 ($CD133^+$) have also been demonstrated to have an important signaling function, attracting and activating cells from the tumor microenvironment (Borovski *et al.*, 2011). Another study suggested that $CD133^+$ melanoma cells have an enhanced ability to initiate primary tumors compared to melanoma cells that not expressed CD133 ($CD133^-$) (Monzani *et al.*, 2007).

Furthermore, CD44, a very well described m-CSCs surface marker, has been a topic of an intense research interest, specially due to the increased evidences of their role in tumor progression and metastasis (Negi *et al.*, 2012; Thapa & Wilson, 2016). The CD44 is a cell molecule adhesion overexpressed in several types of cancers, including melanoma, and might promote the ability of cancer cells to self-renew and differen-

tiate by interacting with tumor microenvironment (Morath *et al*., 2016). Overall years, various studies have been correlated the level of CD44 in malignant melanoma cells lines with a higher metastatic risk (Dietrich *et al*., 1997; Ahrens *et al*., 2001). In addition, it was demonstrated that the stimulation of CD44 by hyaluronic acid on melanoma cells mainly induced an increase in proliferative capacity of these cells (Ahrens *et al*., 2001) . Dou and coworkers (2007) demonstrated that the co-expression of the markers CD44 and CD133 in B16F10 melanoma cells are associated with stronger tumorigenic potential in mice and the identification of these two markers provide an important method for further CSCs target therapy.

Another important marker of mature B cells associated with m-CSCs is the CD20. Fang and collegues (2005) suggested a correlation between CD20 expression in melanoma sphere cultures and preferential clonogenicity capacity. Due to this property, the monoclonal antibody against CD20 (Rituximab®) has been tested aiming m-CSC elimination. Schmidt and collegues (2011) demonstrated that CD20 was an effective target to eradicate estabilished melanoma lesions in immunodeficient mice. Furthermore, clinical studies evaluating metastatic melanoma patients treated with Rituximab® resulted in a regression of metastatic lesions and prevention of disease recurrence (Pinc *et al*., 2012; Schlaak *et al*., 2012).

The CD271, a neurotrophin receptor, has been related with m-CSCs profile (Valyi--nagy *et al*., 2012). This receptor is widely expressed in human normal and neoplastic tissues of neural crest origin, specially melanoma (Kruger *et al*., 2002). Recently, it was suggested an inversely correlation between the CD271 expression in melanoma cells and tumor progression. The expression of CD271 exhibited a significantly decrease in metastatic melanoma cells when compared with primary tumor using *in vitro* zebrafish melanoma model of three-dimensional multicellular spheroids (Saltari *et al*., 2016).

One of the suggested explanations for these results was that CD271 negative profiles promoted the down regulation of β_1-integrin, decreasing the cell-cell adhesion which improved the cells ability to invade and causes the melanoma progression. Another cluster differentiation molecule that also has been correlated with poor prognosis in malignant melanoma patients is the activated leukocyte cell adhesion molecule (ALCAM/CD166). This m-CSC marker is involved in cell growth, migration and adhesion. The regulation of cell adhesion in tumor tissue is a key process for metastatic development through the cell evasion from primary tumor to surrounding tissue (Swart, 2002; Weidle *et al*., 2014).

BIOMARKERS FOR CMCS

Van der Bruggen (1991) discovered the first Melanoma Differentiation Antigens (MDA), proteins that are only expressed on melanocyte lineage, whether normal or tumor cells. Since then, more than 55 proteins with a homolog domain of 200 amino acids (MAGE Homology Domain, MHC) were identified and characterized. These proteins represent the melanoma differentiated cells and are associated with tumorigenic phenotypes (Sang *et al*., 2011). MAGE proteins are rather expressed in normal cells but is overexpressed in various forms of cancers as bladder, breast, squamous carcinoma and more frequently in melanoma and lung cancer (Roeder *et al*., 2005). The recent literature showed that these proteins play a major role in cell cycle progression and apoptosis as also on immune response against cancer. However, their biological functions and mechanisms are not yet well understood (Sang *et al*., 2011).

A well-characterized biomarker of this family is the MAGE-A3, a tumor-specific antigen expressed in a variety of cancers and presented in 57% to 76% of metastatic melanoma. It has been utilized as a diagnosis and prognosis biomarker for CMC and has also been studied as a target for cancer immunotherapy (Sigalotti *et al*., 2002; Roeder *et al*., 2005). Despite of MAGE-A3 has been considered an attractive target for immunotherapy, this antigen recently failed in two different phase 3 trials for melanoma and non-small-cell lung cancer (NSCLC). The adjuvant treatment with the MAGE--A3 immunotherapeutic did not increase disease-free survival and any other clinical outcome measure compared with placebo (Vansteenkiste *et al*., 2016). These results reinforce the problematic of cancer vaccination technology to improve the prognostics in patients and overcome the immunosuppressive environment of aggressive cancer types as NSCLC.

Apart MAGE antigens, another three MDA can be highlighted, especially due to the application in melanoma diagnosis and cancer immunology: melanoma antigen recognized by T-cells (MART-1/Melan-A); glycoprotein 100 (gp100) and tyrosinase (TYR). These melanocyte antigens are responsible for melanoma differentiation, biosynthesis of melanin and T-cells recognition of antigens presented at cells surface. In recent years, a new approach for cancer prevention and immunotherapy are the development of vaccines using these antigens, specially MART-1 (Gibney *et al*., 2015; Reed *et al*., 2015; Tazzari *et al*., 2015).

The melanocyte differentiation marker MART-1 is found in the membranes of the Golgi apparatus, endoplasmic reticulum, as well as the plasma membrane itself (Chen *et al*., 1996; De Mazière *et al*., 2002). It is homogenously expressed in normal melanocytes from skin and retina and in 75 to 100% of human melanomas, but not in

other cancer types (Meng *et al*., 2015). Mockey and collegues (2007) developed histidylated lipopolyplexes containing MART-1 mRNA and demonstrated that this system was effective on protected against B16F10 melanoma tumor progression, drastically reducing by 75% the total number of lung metastases. A pilot phase I-II trial designed by Pucchio and collegues (2006) evaluated the effects of a co-therapy using IFN-α; Melan-A/MART-1 and gp100 peptides in stage IV melanoma patients. It was demonstrated an enhancement in CD8+ T cells recognizing MART-1+gp100+ melanoma cells.

TYR, a protein expressed in melanocytes, is another important CMC biomarker utilized in cancer immunotherapy. The technique of RT-PCR can detect marker RNA expression in the peripheral blood and was used for the first time by Smith, Lattman & Carter (1991) for detection of TYR. Cancer vaccines based on injection of xenogeneic TYR DNA peptide have been tested and demonstrated to induce humoral and cytotoxic lymphocyte immune responses against human melanoma cells that express TYR, resulting in tumor growth inhibition (Yuan *et al*., 2013). On the other side, the most relevant achievement for xenogeneic TYR DNA vaccine have been the effectiveness of this tumor associated antigen to improve survival in dogs with metastatic melanoma (Bergman *et al*., 2006; Aurisicchio *et al*., 2015). Based on these positive results, xenogeneic TYR DNA vaccine (Oncept®) was commercially approved in USA (Bergman *et al*., 2006).

In the context of MDA, various researches have been observed an upregulation of gp100, MART-1 and TYR antigens in melanoma cell lines treated with (v-raf murine sarcoma viral oncogene homolog B1) (BRAF) and mitogen-activated protein kinase kinase (MEK) inhibitors, resulting in improvement of antigen-specific recognition by gp100 and MART-1 specific T-cells (Boni *et al*., 2010; Ott *et al*., 2013). It has been suggested that the oncogenic BRAF suppressed the MDA expression by Microphthalmia Associated Transcription Factor' (MITF). This transcriptional factor is also considered a class of human melanoma marker that regulates the transcription of multiple MDAs. MITF is consider the master regulator of melanocyte development and melanoma oncogene. It is also involved with the plasticity of melanoma cells (Hartman *et al*., 2014). The overexpression of this oncogene MITF was shown high sensitivity for metastatic melanoma (88-100%) and could be associated with a reduced survival in melanoma patients (Prieto & Shea, 2011). MITF can also support the diagnosis of metastatic tumors that are suspicious for melanoma but negative for common melanoma markers as MART-1 and TYR (Guo *et al*., 2013).

Melanocortin-1 receptor (MC1R) is an important member of G-protein-couple receptor family that regulates the amount and type of melanin (eumelanin and pheomelanin) produced from melanocytes, which determine the melanoma phenotype and

risk factor. On the other hand, the expression and function of MC1R in amelanotic and nonmelanocytic tumors remains unclear (Ghiorzo *et al*., 2009; Ordóñez, 2014). This receptor is highly expressed in melanoma but lower expressed on normal cells and other cancer types (López *et al*., 2007). Kennedy and collegues (2001) described that numerous MC1R variants predispose to cutaneous melanoma and this predisposition is largely independent of skin type. It is also suggested that the risk for malignant melanoma, associated with MC1R variants, was confined only to BRAF-mutant melanomas (Fargnoli *et al*., 2008).

Despite of above discussed evidences, the recent literature about the feasibility of CMCs clinical applications demonstrated to be controversial. Although there was a general agreement that correlated the abundance of CMCs tumor biomarkers in blood flow with a poor prognostic and decrease of patients overall survive another researches suggested that the use of melanocytic markers to detect CMCs could lead to false-negatives results, specially with the cells that presents anmelanotic and phenotypes associated with lack of pigmentation production (Notani *et al*., 2002).

One of the most used methods for CMCs isolation is based on the immunocytochemical identification of surface markers (Liu *et al*., 2011). The CellSearch® system is a recent platform commercially approved by FDA for CTCs isolation. This technique is based on targeting cell markers in metastatic cancers, such as epithelial cell adhesion molecule (EpCAM) and melanoma cell adhesion molecule (MCAM/CD146/MUC18) (Farace *et al*., 2011). MCAM, generally expressed in lymphoid tissues as a receptor for laminin alpha 4, is strongly expressed on the surface of CSCs derived from human bone marrow (Covas *et al*., 2008; Russell *et al*., 2013). In addition, this receptor is also largely expressed by endothelium cells and their function has been associated with support of endothelial integrity (Schrage *et al*., 2008). Besides, the MCAM is up-regulated in inflammatory diseases and is also involved in lymphocyte recruitment by endothelium (Guezguez *et al*., 2007; Duan *et al*., 2013). Despite the lack of specificity for melanoma, studies have been explored MCAM as a promising target in melanoma diagnosis and cancer therapy, particularly in cases where the histology is suggestive but other melanoma markers are negative (Koch *et al*., 2001; Staquicini *et al*., 2008). Besides, recent studies associated the detection of MCAM/MUC18 in melanoma patients as a molecular warning of melanoma metastatic potential, with higher incidence of disease relapse, poor prognosis and death (Elshal *et al*., 2005; Rapanotti *et al*., 2014).

Biomarkers also associated with general tumorigenic phenotypes have been used for target melanoma diagnosis and treatment. Two examples of these types of general makers are the Ganglioside GD2 and HMW-MAA/CSPG4. The Ganglioside GD2 is a membrane receptor, highly expressed on tumors of neuroectodermal origin as

melanoma, neuroblastoma, brain tumors and osteosarcomas, and have restricted expression in normal tissues, specially in peripheral nerves, melanocytes and brain cells (Longee *et al*., 1991; Yu *et al*., 2016). This ganglioside receptor is also highly expressed in human mesenchymal stem cells and has been reported as a useful cancer stem cells biomarker, specially for neuroblastomas, breast cancers and melanoma (Battula *et al*., 2012; Senses *et al*., 2017). Studies suggested that the anti-GD2 antibodies can have a direct cytotoxic activity, inducing a rapid cell death when incubated with GD2-positive tumor cells (Kowalczyk *et al*., 2009; Doronin *et al*., 2014). Multiple clinical trials have been performed using different types of anti-GD2 monoclonal antibodies classes in different cancer types, including melanoma (Albertini *et al*., 1997; King *et al*., 2004; Choi *et al*., 2006). Generally, the positive results of these studies were prominent to neuroblastoma cases, improving patients survival (Handgretinger *et al*., 1995; Cheung *et al*., 1998; Navid *et al*., 2014). However, the treatment with anti-GD2 antibodies have been related with peripheral nerves fibers toxicity resulting in acute pain during the treatment (Roth *et al*., 2014). Phase I trial using humanized Anti-GD2 is ongoing in children and adolescents with neuroblastoma, osteosarcoma, ewing sarcoma and melanoma (ClinicalTrials.gov identifier: NCT00743496). Recently, FDA has approved the antibody GD2, dinutuximab®, for the treatment of pediatric patients with high-risk neuroblastoma, based on findings from a phase III clinical trial conducted by the Children's Oncology Group (Yu *et al*., 2010).

Another well-characterized melanoma surface antigen is the melanoma-associated chondroitin sulfate proteoglycan or high molecular weight-melanoma-associated antigen (CSPG4/HMW-MAA/NG2). This transmembrane proteoglycan is frequently expressed on normal tissues throughout development and in various types of cancers, including glioma, squamous cell carcinoma, breast carcinoma and melanoma. In addition, CSPG4 is expressed by cancer stem cells in squamous cell carcinoma, glioblastoma, breast carcinoma and melanoma (Major *et al*., 2013; Beard *et al*., 2014). In fact, studies have been demonstrated that CSPG4 plays an important role in controlling tumor microenvironment signals, specially through the activation of integrins promoting adhesion, motility and survival of cancer cells (Bluemel *et al*., 2010). CSPG4 protein is expressed in all melanoma stages, probably due to their multifunctional mechanisms that regulates multiple oncogenic pathways which leads the melanoma progression, enhancing the metastatic properties (Burg *et al*., 1998). Therefore, the CSPG4 has been considered as a promising immunotherapeutic target to delaying progression and/or recurrence in melanoma patients (Wang *et al*., 2011).

CHAPTER 4

STRATEGIES FOR METASTATIC MELANOMA TREATMENT

SURGERY

In general, surgery is the first-line treatment for the excision of localized melanoma primary tumours (Dummer *et al*., 2015). Related to cutaneous melanoma metastasis, the surgical resection of metastasis lesions in the same region or outside the region of the primary tumor, demonstrated to be a promising palliative option, when combined with systemic therapy, to improve the patient survival rates (Sosman *et al*., 2006; Agarwala *et al*., 2014; Maverakis *et al*; 2015). Nevertheless, the effectiveness of surgical excision for advanced melanoma patients depends on the number of resectable metastasis sites and the surgery is only indicated, except for palliation, when the melanoma has spread to only limited sites, the most communs including the lungs, lymph nodes, brain and liver (Leung *et al*., 2012).

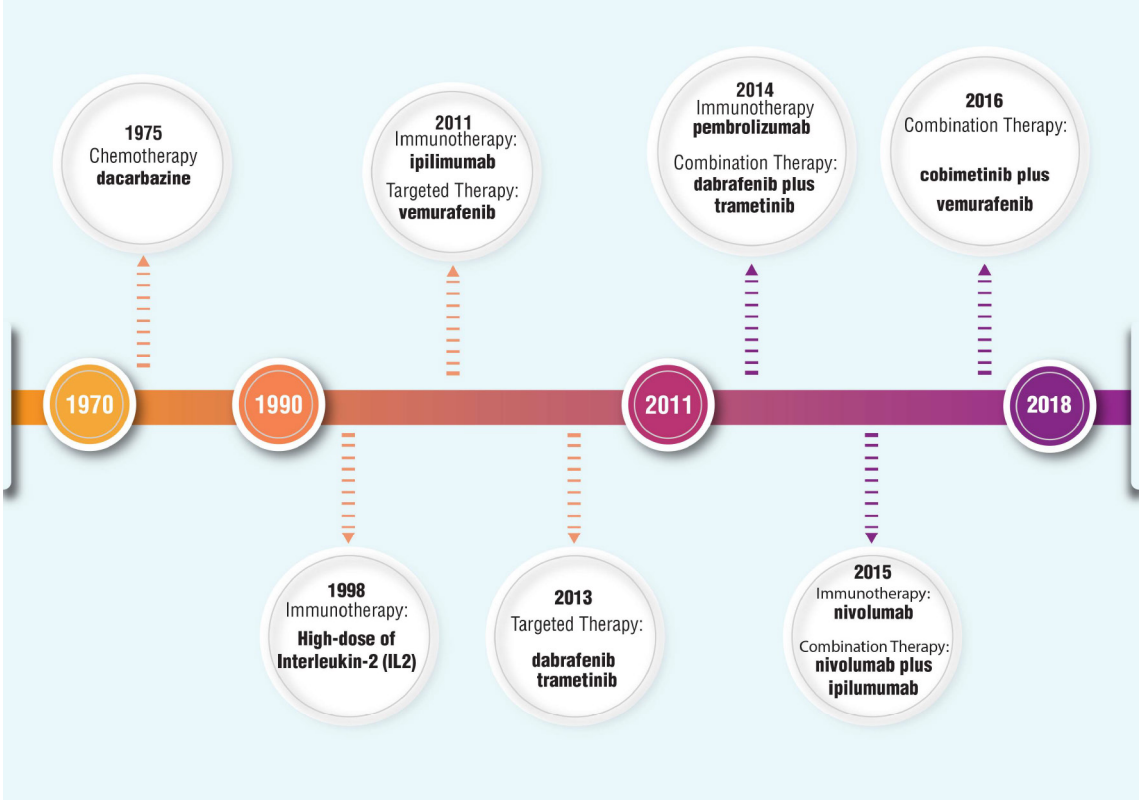
MONOTHERAPY AND COMBINATION THERAPY

Over 30 years, one of the most used biochemotherapic approaches for treatment of metastatic malignant melanoma consisted in the monotherapy with the administration of classical chemotherapeutic agents such as dacarbazine (FDA, 1975) and immunotherapeutic agents such as interferon-alfa-2b and interleukin-2 (Legha *et al*., 1998; Rosenberg *et al*., 1999). However, several studies demonstrated that only a small part, below 20%, of advanced metastatic melanoma patients treated with these agents, separately or in combination, had a relevant impact on five-year survival rates or clinical regression (Maio *et al*., 2015). In addition, serious side effects have been associated with conventional chemotherapeutic agents specially due to high toxicity to normal cells, low bioavailability, non-specific distribution and multidrug resistance (Gao *et al*., 2014).

Many efforts have been made, over the past decade, to overcome these drawbacks, improving the efficacy of classical chemotherapeutic agents and introducing targeted therapies and immunotherapies (Amann *et al*., 2016; Kakavand *et al*., 2016). The recent anti-melanoma agents approved by FDA for monotherapy or combination therapy regimens, including: the second-generation BRAF inhibitors, vemurafenib and dabrafenib (Chapman *et al*., 2011; Hauschild *et al*., 2012), the MEK inhibitors, trametinib and cobimetinib (Kim *et al*., 2012; Larkin *et al*., 2014) and the immunotherapeutic agents, ipilimumab, pembrolizumab and nivolumab (Hodi *et al*., 2010; Robert *et al*., 2014; Wolchok *et al*., 2013) (Figure 2). The monotherapy with these anti-melanoma agents represented a great medical breakthrough, leading to better prognostics to advanced melanoma patients, with a clinically meaningful survival benefit on order of 5-7 months for targeted agents, such as vemurafenib and trametinib (Weber *et al*., 2016; Najem *et al*., 2017). Nevertheless, the single-agent therapy for metastatic melanoma pa-

tients demonstrated to present several drawbacks, including high tumor relapse rates, drug resistance, low percentages of cure and serious toxic effects (Topalian *et al*., 2014; Larkin *et al*., 2015; Grob *et al*., 2015).

Figure 2 - Milistones of metastatic melanoma treatment, drugs approved by FDA until present.



Source: Authors.

On the other hand, the combination therapy, with immunotherapeutic and targeted agents, demonstrated to achieve a long-term prognosis, fewer side effects and high efficacy compared to monotherapy; prevent and/or overcome resistance (Long *et al*., 2014; Grob *et al*., 2015; Ascierto *et al*., 2016; Wolchok *et al*., 2017). Despite of the promising results of combination therapy, most patients with advanced melanoma until presents drug resistance and severe toxicities (Long *et al*., 2014; Moriceau et ak., 2015; Fattore *et al*., 2017; Keller *et al*., 2017). It has been reported that 67% of the advanced melanoma patients presented an acquired resistance to anti-PD-1 therapy and the median time to resistance was 11 months (Gide *et al*., 2017; Wang *et al*., 2017). Related to the toxicity of combination immunotherapy, the most relevant can be exemplified as immune-mediated diarrhea, pneumonitis, colitis, hepatitis, hypophysitis, keratoacanthomas, squamous cell carcinoma, respiratory toxicity, arthralgia, thyroid disorders and hypotension, which leads to a treatment discontinuation or multiorgan failure and death of approximately 30% of patients (Ma & Armstrong, 2014; Chuk *et al*., 2017; Sznol *et al*., 2017). In view of these therapeutic issues, is necessary to investigate the

suitable timing and sequences of combination regimens that can improve the efficacy and decrease the toxicity of the treatment (Najem te al., 2017).

RADIOTHERAPY

Another therapeutic strategy used in advanced melanoma patients is the radiotherapy (RT). Radiotherapy can provide palliation for the patients who develop unresectable, locally recurrent, or symptomatic metastatic disease, especially in intracranial metastases (Maverakis *et al*., 2015; Kyu Hwang *et al*., 2017). Most recently, preclinical and clinical studies of RT combined with immunotherapeutic agents in the treatment of advanced melanoma have been reported (Chandra *et al*., 2015; Liniker *et al*., 2016). The ability of RT and immunotherapy to increase the immune-response to tumors located in distant non-irradiated metastatic sites is called abscopal effect. This event has been related with an improvement of antigens presentation and expansion of T lymphocytes, recognizing melanocyte specific antigens, which contributes to the increase of therapeutic response to immunotherapy (Okwan-Duodu *et al*., 2015; Demaria *et al*., 2015). On the other hand, the potential toxicity, the dose and regimen of the combined RT and immunotherapy remains a concern and more studies should be performed to improve the quality of safety data of this therapeutical approach (Liniker *et al*., 2016). Recently, clinical trials reported several toxicities related to the combination of anti-PD-1 antibodies and radiotherapy in patients with metastatic melanoma, including radiation dermatitis, cerebral edema, lymphoedema and neurotoxicity (Henderson *et al*., 2015; Liniker *et al*., 2016).

VACCINES

More recent approach for metastatic melanoma treatment consist in the development of vaccines that aims to augment the recognition by cytotoxic T lymphocytes (CTLs) of specific antigens presented by melanoma cells (Chung *et al*., 2017; Zhang *et al*., 2018). This strategy can be complementary to the therapy with immune checkpoint inhibitors, such as ipilimumab, leading to an immune-mediated tumor regression, improving clinical responses (Ali *et al*., 2016; Stark *et al*., 2017). Despite of the promising pre-clinical results, melanoma vaccines have demonstrated a limited therapeutic effect and high toxicity in clinical studies, probably due to the intrinsic tumor heterogeneity that limits the effective presentation of tumor antigen and leads to poor recognition by the immune system (Lowe *et al*., 2013; Ott *et al*., 2017; Ferguson *et al*., 2018).

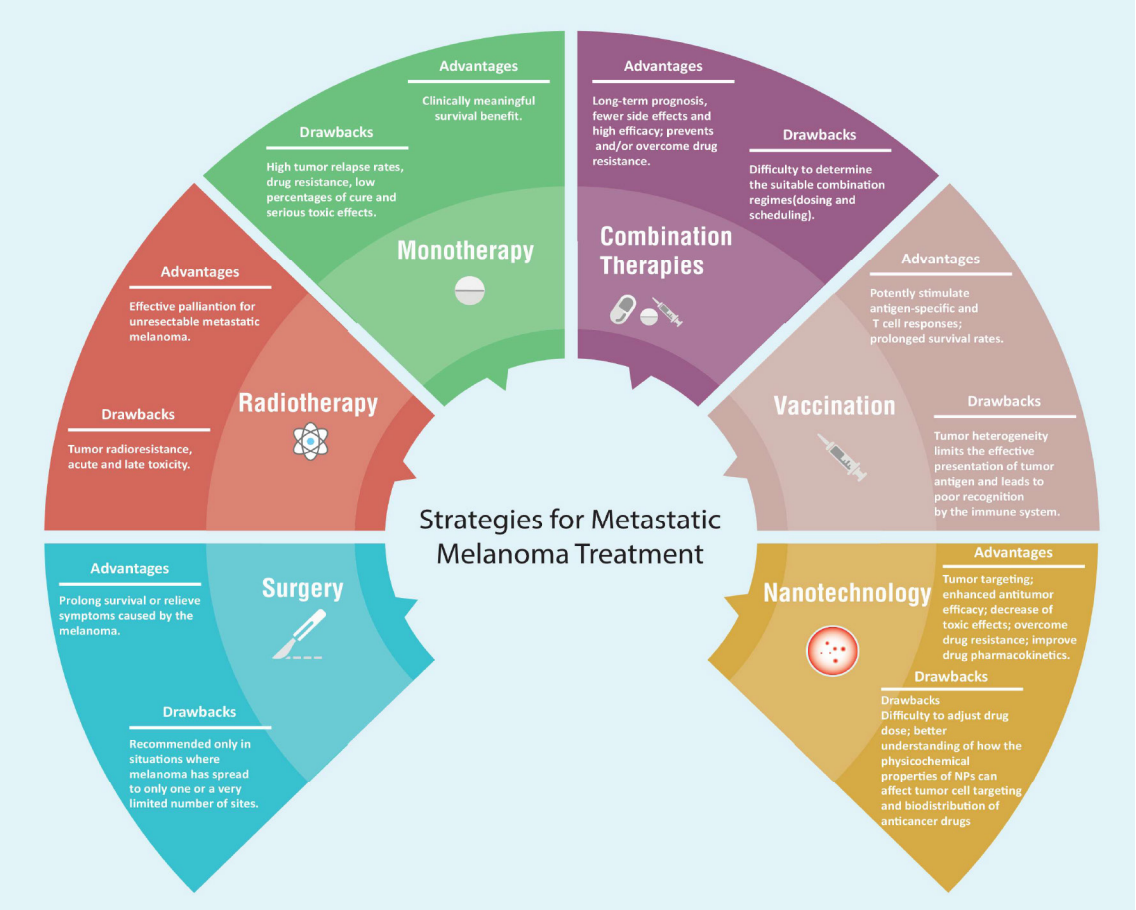
NANOTECHNOLOGY

In general, nanotechnology is a multidisciplinary field and can be defined as the engineering and manufacturing of materials at the atomic, molecular or supramole-

cular scales with the aim to produce systems with specific and unique characteristics (Maynard, 2006). In biomedical field, specially in oncology, the application of nanotechnology in drug delivery systems has been extensively explored through the development of nanoscale-sized structures for local drug delivery (Xie *et al*., 2015; Piktel *et al*., 2016). Among the different types of nanoscaled drug delivery systems, the most studied for anticancer applications are lipid-based or polymer-based nanoparticles (Prabhu *et al*., 2015; Arranja *et al*., 2017).

In view of these advanced melanoma treatment approaches, the nanotechnology has been considered one of the most important strategies to overcome the hazards related to the current cancer therapies, such as toxicity, limited efficacy and/or drug resistance. In this way, nanotechnology approach aiming to improve the efficacy of existing cancer therapies, promote the site specificity on tumor cells and minimize the several adverse effects arising from off-target toxicities. Besides anticancer drug delivery applications, nanocarriers have been extensively used to incorporate imaging agents in multi-functional nanoparticles and improve their biodistribution to cancer sites allowing the monitoring of disease progression in real-time (Daga *et al*., 2016; Parvanian *et al*., 2016). Furthermore, the integration, in a single formulation, of therapeutic drug delivery and diagnostic agents, characterizing a theranostic nanomedicine, has been considered a promising strategy to personalize the cancer treatment and to avoid the metastasis through early diagnosis and continuously monitoring of therapeutic response (Sharma *et al*., 2016; Shi *et al*., 2016). However, some drawbacks related to the nanotechnology applications for advanced melanoma treatment including the difficulty to adjust drug dose and a better understanding of how the physicochemical properties of nanocarriers can affect tumor cell targeting and biodistribution of anticancer drugs need to be overcome (Bertrand *et al*., 2014; Chen *et al*., 2017). The Figure 3 resumes all these discussed strategies for metastatic melanoma treatment.

Figure 3 - Strategies for metastatic melanoma treatment: their advantages and related drawbacks.



Source: Authors.

CHAPTER 5

RESISTANCE MECHANISMS OF MELANOMA CELLS, M-CSCS AND CMCS

As already discussed in previous sessions, the subpopulation of m-CSCs and CMCs play a key role in development of distant metastasis and their presence are correlated with poor prognostics. The multidrug resistance of m-CSCs to currently chemotherapy agents is until one of the major therapeutic challenge in advanced melanoma treatment and can explain the high incidence of disease relapse, giving rise to new tumors and metastases (Vinogradov & Wei, 2012). In general, CSCs demonstrated an enhanced capacity to develop specific drug resistance mechanisms to chemotherapy, such as the overexpression of different drug efflux transporters (Abdullah & Chow, 2013). Various drug efflux transporters involved in chemoresistance of m-CSCs have been identified, including P-glycoprotein (P-gp), DNMT3B, EPAS1, JARID1B, TERT and ABC multidrug transporters, specifically ABCB5, ABCB1 and ABCG2 glycoproteins (Wouters *et al*., 2013; Wilson *et al*., 2014).

Other factors as the presence of antiapoptotic signaling pathways, specific protective microenvironment and hypoxia are responsible for the multiple resistance mechanisms of CSCs in tumor site and in blood flow. The most extensively characterized growth and survival pathway involved in melanoma resistance to apoptosis is the phosphatidylinositol-3-kinase (PI3K) pathway (Paluncic *et al*., 2016). The activation of PI3K results in phosphorylation of ERK and protein kinase B (AKT) leading to an activation of the mammalian target of rapamycin (mTOR) and GSK3β inhibition, respectively. In turn, the inhibition of GSK3β protein results in an upregulation of oncogenic genes, such as c-MYC and cyclin D1, that leads to a strong anti-apoptotic effect and cancer progression (Brachmann *et al*., 2009). Studies demonstrated that inhibition of survival mechanisms as PI3K/m-TOR pathway could overcome melanoma acquired resistance to MAPK inhibitors (Kolev *et al*., 2014; Vaidhyanathan *et al*., 2016). In addition, another known CSCs antiapoptotic resistance mechanism in melanoma is the dysregulation of BCL-2 family members. Combination strategies to BCL-2 targeting have been demonstrated to be efficient in eliminating both wild-type and mutant BRAF melanoma cells and m-CSCs (Mukherjee *et al*., 2015).

Still in this context, one of the major characteristics that contributes for m-CSCs multidrug resistance is the existence of a protective microenvironment with specific properties that helps to maintain the m-CSC in a quiescent state and consequently minimizing the chemotherapy effects (Vinogradov & Wei, 2012). The phenotypic plasticity of melanoma cells explains how the cells respond to microenvironmental signals that downregulates the melanocytic proliferation activity and activates a mesenchymal cell state which conduce to a more metastatic potential (Widmer *et al*., 2015). The niche-associated vasculature supports, protects and maintain the CSCs and the heterogeneous microenvironment composed by different cell types and cytokines (Vinogradov & Wei, 2012). Considering these characteristics, the combination of antian-

giogenic therapies and chemotherapies can reduce the number of CSCs and increase the tolerance to chemotherapy toxicity (Spitler *et al*., 2015; Haase *et al*., 2016). On the other hand, besides the preclinical studies and clinical trials demonstrated that antiangiogenic agents have a potential efficacy to suppress tumor growth, several studies have been suggested a limited survival benefit, high relapse rates, acquired drug resistance and toxicity (Pàez-Ribes *et al*., 2009; Gacche & Meshram, 2014). These drawbacks are specially related to the fact that the anti-angiogenic agents can target indiscriminately both physiological and pathological angiogenesis resulting in toxicity and limiting efficacy due to compensatory angiogenesis pathways/revascularization (Wang *et al*., 2016).

Hypoxia has been considered one of the most important triggers to induce phenotype switch of proliferative melanoma cells to cancer mesenchymal cells with more invasive characteristics, capable to survive and proliferate in low oxygen ratio conditions. A well-known protein that mediates the hypoxic response is the HIF1alpha, more expressed in aggressive melanoma subtypes (Marconi *et al*., 2015; Rhee *et al*., 2016). In response to the hypoxia, this protein regulates the expression of transcriptional genes that codify proangiogenic factors involved in angiogenesis induction and apoptosis regulation, sustaining the tumor progression due to physiological adaptation to a low oxygen tension (Jour *et al*., 2016).

Other important reported consequences after treatment with angiogenesis inhibitors described in clinical assessments is the development of more invasive-metastatic phenotypes (Haase *et al*., 2016; Jayson *et al*., 2016). *In vivo* studies demonstrated the approved antiangiogenic agents, sunitinib and sorafenib, can facilitate metastatic dissemination of syngeneic melanoma in mice (Ebos *et al*., 2009; Pàez-Ribes *et al*., 2009). The researchers suggested that the typical plasticity phenotype of CSCs and the capacity to survive to hypoxia conditions makes the cells resistant to angiogenesis inhibitors, which also can explain the aggressive recurrence of tumors and adaptive resistance after treatment (Gacche & Meshram, 2014).

To overcome these consequences, studies suggest that the combination therapies associated to the nanotechnology could reduce the stem cell-associated drug resistance and enhance the chemotherapeutic efficacy (Mukherjee & Ranjan, 2016).

CHAPTER 6

NANOTECHNOLOGY APPROACH TO MELANOMA CELLS, M-CSCS AND CMCS TARGETING

In a review of Brys and collegues (2016), they emphasize how the nanotechnology-based strategies provides an opportunity to vanquish drug resistance and toxicity associated with current advanced melanoma therapies and improving pharmacokinetics, targeting, or other features of anti-cancer pharmaceuticals. It is already well established that the most efficient strategy to prevent the multidrug resistance in advanced melanoma patients is the combination therapy. The combination of different pharmacotherapies for metastatic melanoma as kinase inhibitors, immunomodulators and conventional chemotherapeutic agents as paclitaxel (PTX), could reach a greater number of potential targets involved in melanoma development and consequently result in a higher overall response and progression free survival (Bombelli *et al*., 2014; Brys *et al*., 2016).

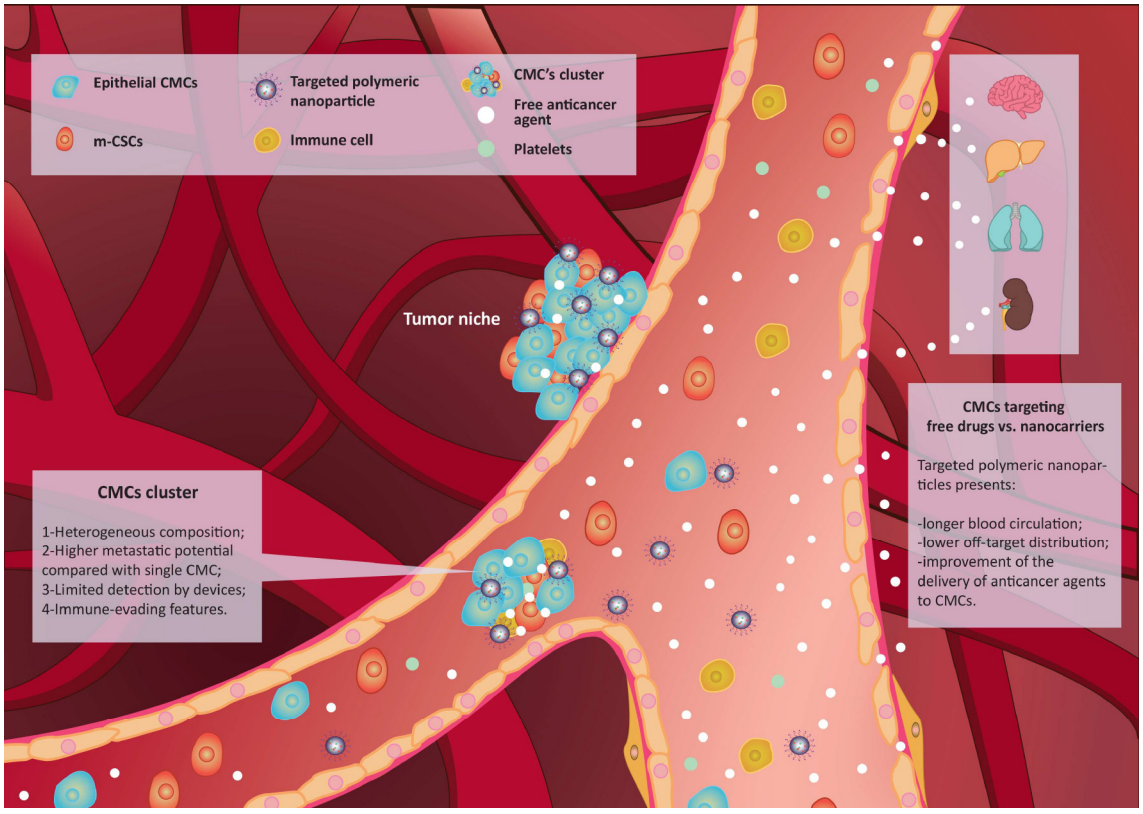
Several studies have been demonstrated that the vehiculation of antiangiogenic agents in nanoparticles can surpass the drug resistance, toxicity and low efficacy relative to the pure drug (Guan *et al*., 2014a; Guan *et al*., 2014b; Mukherjee *et al*., 2015; Haase *et al*., 2016). It is already well established that the tumor vascularity is critical to regulate tumor microenvironment functions and consequently ensure CSCs survival. A strong correlation was observed between enhanced tumor growth and metastasis in human malignant melanoma and vascular endothelial growth factor (VEGF) overexpression (Spitler *et al*., 2015). Strategies to indirectly eradicate CSCs by encapsulation of cytotoxic and antiangiogenic agents in nanoparticles, including VEGF antibody (bevacizumab), have been reported (Guan *et al*., 2014a; Guan *et al*., 2014b). The antibodies encapsulation into nanoparticulated delivery systems can decrease the dosage, toxicity and treatment cost, besides enhanced efficacy.

Other promising anti-cancer molecules candidates to nanotechnology applications is efflux pump and/or antiapoptotic inhibitor. Drug delivery systems encapsulating these inhibitors have been a promising approach to increase the bioavailability and consequently the therapeutic efficacy of wide range of anti-cancer drugs, specially their target to CSCs (Chen *et al*., 2014; Wu *et al*., 2017). Related to antiapoptotic effect, the nanotechnology can be applied to improve the inhibition of PI3K/AKT pathway by using iron and zinc oxide nanoparticles that demonstrated to induce cytotoxicity and apoptotic death in hepatocytes and macrophages (Sarkar & Sil, 2014). In a recent research, the co-encapsulation of two drug resistance inhibitors, celecoxib to down-regulate P-gp efflux pump and buthionine sulfoximine that inhibit glutathione synthesis, into polymer/inorganic hybrid nanoparticles demonstrated to be promising strategy to reverse drug resistance in tumor treatments. In this study, was observed a significant improve in tumor cell inhibition after resistant cancer cells were treated by doxorubicin-loaded nanoparticles, indicate that the dual-inhibitor co-delivery system can effectively reverse drug resistance (Wu *et al*., 2017).

ADVANTAGES AND CHALLENGES OF NANOTECNOLOGY-BASED SYSTEMS TO CTCS/CMCS TARGETING

Less than 1 in 10000 circulating tumor cells can survive to blood system, however merely one cell is enough to metastasize other tissues, decreasing the patient's overall survival. The rarity of these cells in circulation make their early blood detection a great weapon to improve the patient's prognostics, assessing tumor progression or even avoid metastasis and cure cancers (Wang, 2016). In this scenario, the use of nanotecnology for CTCs targeting can offer several advantages over conventional treatments based on free drugs (Figure 4). It is well-described in literature that the encapsulation of free drugs on nano-based systems can reduce the clearance and increase the plasma drug exposure, enhancing the apparent drug circulation half-life (Kadam *et al.*, 2012; Blanco *et al.*, 2016). These pharmacokinetic features are required for the efficacy of nanocarriers that aims to intercept CTCs in blood, avoiding possible metastasis and minimizing side-effects when compared to their free drug counterparts (Blanco *et al.*, 2016; Yao *et al.*, 2017). Moreover, nano-based sytems holds a wide range of functionalization possibilities (e.g. stealth, targeting and stimuli-responsive groups) and can combine different therapeutic and diagnostic strategies into one nanosystem (Hu *et al.*, 2015; Yao *et al.*, 2017).

Figure 4 - Characteristics of CMCs clusters and the use of free drugs and nanocarries for CMCs targeting.



Source: Authors.

Nevertheless, nanotechnology applications for detection and treatment of the small population of circulating cancer cells, including melanoma cells, remains to be an enormous challenge. Nanocarriers must face several different biological barriers and obstacles including hemorheological limitations and pressure gradients which can limit their site-specific bioavailability and recognition by the rare population of CTCs in blood flow (Blanco *et al*., 2016). Accordingly, researches involving CTCs-targeting by nanoplataforms are still incipient and require the development of suitable methods to mimetize the bloodstream shear stress conditions and identify the physicochemical parameters that influence cell-particle interactions (Chen *et al*., 2017). Recently, an *in vitro* study reported the use of a cone-and-plate viscometer to mimics the venous flow viscosity, based on previously platelet activation tests, which was able to analyze the cellular uptake of nanoparticles in a fluidic state (Lu *et al*., 2013; Yao *et al*., 2017). In another *in vitro* study using melanoma CTCs, a flow system was used to mimic the circulatory system with shear stress regulated by a peristaltic bomb (Chen *et al*., 2017). However, no currently widely validated method for this type of analyzis and evaluation of cellular uptake using flow conditions was available.

Despite of these challenges, the therapeutic and/or diagnostic use of nanotecnology-based systems for the interception and/or neutralization of CTCs in the bloodstream have been described in the literature. Examples of recent developments in nanotechnology to detect CMCs in blood flow for diagnosis purposes include: Raman scattering (SERS) nanoparticles (Wu *et al*., 2016); Cross-linked iron oxide nanoparticles conjugated with melanocyte markers, such as MART-1 (Gee *et al*., 2016); MCR1 antibody immobilized in amino-functionalized silica nanoparticles (Seenivasan *et al*., 2015) and also a poly(lactic-co-glycolic acid) (PLGA)-nanofiber nanovelcro chip conjugated with melanoma-specific antibody as anti-CD146 (Hou *et al*., 2013). Related to the targeting of CTCs in the bloodstream for treatment purposes, a recent study of Yao and coworkers (2017), using a metastatic breast cancer mice model, developed a dual-targeting polymeric nanoparticle for antiangiogenic therapy and CTCs interception. This study demonstrated that the multifunctional nanoparticles exhibited a potent anticancer effect and the double target act in synergism against the highly invasive breast cancer. These results are very encouraging for future research involving CTCs targeting nanoparticles and other metastatic cancer models.

In view of the discussion above, the use of nanotechnology can be a useful tool to combat the chemotherapy resistance mechanisms developed by m-CSCs/CMC and the inherent disadvantages of currently available treatments options, improving the effectiveness of anti-cancer drugs (Banerjee *et al*., 2011; Burke *et al*., 2012). The hypothesis that supports the nanomedicine therapeutic approach to specifically targeted m-CSCs/CMC is based on the harness potential of nanotechnology to create modifia-

ble drug-delivery platforms, capable to carry high payloads of anti-cancer drugs and increase their uptake by specific cells. Among the wide range of nanomaterials used for this aim, the polymeric nanoparticles are suitable structures for drug transport (Garcia-Mazas *et al*., 2016; Silva *et al*., 2016).

POLYMERIC NANOPARTICLES IN MELANOMA TREATMENT

Biocompatible and resorbable polymers were first introduced in biomedical field as an alternative to metal surgical devices and implants (Ramakrishna *et al*., 2001). Since then, several types of polymeric nanomedicines have been largely studied for anti-cancer therapy and diagnosis, including polymer–lipid hybrid systems (Rao & Prestidge, 2016), micelle-polymeric nanoparticles (Li *et al*., 2015) and polymeric nanoparticles (Li *et al*., 2016; Antônio *et al*., 2017). Nanoparticles formulated with biocompatible and biodegradable polymers are one of the most investigated vectors for cancer therapy, mainly due to these potentially modifiable physicochemical properties and large variety of anti-cancer compounds that can be delivered into tumors in a more specific and homogeneous way (Prabhu *et al*., 2015; Li *et al*., 2016; Vauthier & Ponchel, 2016).

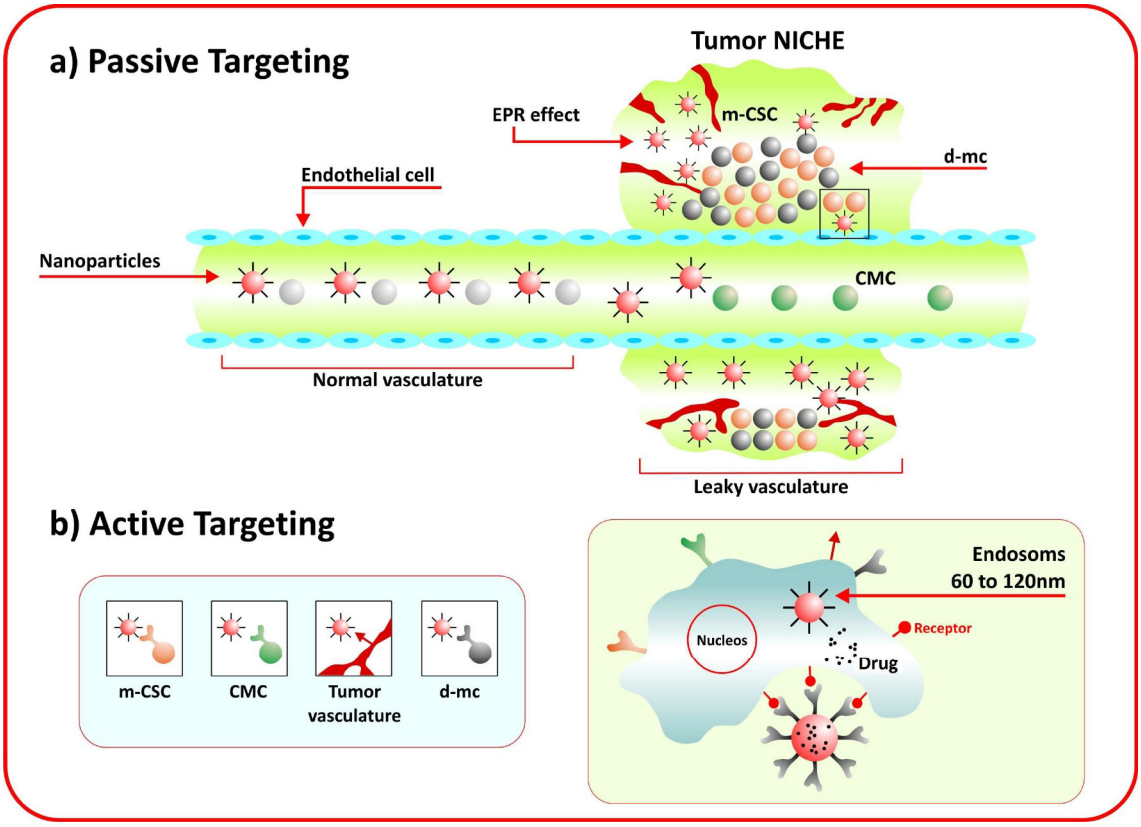
Generally, polymers used to develop nanoparticles are based on polyesters, such as poly (lactic acid) (PLA), poly (glycolic acid) (PGA), polycaprolactone (PCL) and their copolymers poly (alkyl cyanoacrylate) polycarbonates, poly (aminoacids) and polyphosphoesters, and also naturally occurring biodegradable polymers as chitosan and hyaluronic acid-based polymers (Jin *et al*., 2012; Abruzzo *et al*., 2016; Vauthier & Ponchel, 2016).

Among the several advantages of polymeric nanoparticles in cancer therapy and diagnosis the most representatives are: improve solubility and stability of anti-cancer drugs, delivery large doses of chemotherapy agents, promote the accumulation of drugs in tumor site by passive and active targeting, prevents drug leakage and reduce nonspecific biodistribution, reduce toxicity and systemic side effects related to off-target distribution, reduce cancer cell drug resistance, control the drug pharmacokinetics by sustained release, increasing drug circulation time in blood, reduce dose regimens, combinate therapy and imaging agents in a single carrier, targeting multiple pathways in cancer, protect the active principals from enzymatic degradation and rapid clearance *in vivo* (Couvreur & Vauthier, 2006; Prabhu *et al*., 2015).

In the scope of nanoparticles targeting, passive targeting and active targeting are the two main strategies currently used (Liu *et al*., 2014; Kamaly *et al*., 2016), as represented in Figure 5. Each approach takes in account nanoparticle's size, shape and surface charge, tumor microenvironment and cells characteristics (Bazak *et al*., 2014). Among

these nanoparticles, different types have been studied for diagnostic and treatment of advanced melanoma including conventional surface nonmodified; stealth; targeted; pH sensitive and core-shell nanoparticles (Table 2).

Figure 5 - Schematic illustration of passive targeting (a) and active targeting (b) for melanoma treatment and diagnosis. In passive targeting, the enhanced permeation and retention (EPR) effect allows the accumulation of nanoparticles at the heterogeneous tumor niche that contains differentiated melanoma cells (d-mc) and m-CSCs (a). In active targeting, the targeted nanoparticles can recognize surface receptors expressed by m-CSCs, CMC, endothelial cells of tumor vasculature and/or d-mc and promote a receptor-mediated endocytosis and drug-delivery of antitumoral or diagnosis agents into the melanoma cells (b).



Source: Authors.

Table 2 - In vivo and in vitro studies with polymeric nanoparticles loaded with different drugs for passive and active tumor targeting for advanced melanoma treatment.

Polymer(s)	Drug delivered	Systems/Composition	Characteristics	Main results	Reference
PCL	Acetyleugenol (AcE)	AcE-LNCs	Ø ≈210 nm PDI ≈ 0.1 ζ ≈ −10 mV spherical	Oral LNC treatment was more efficient than AcE-LNC treatment B16F10 melanoma tumor model.	Drewes *et al.*, 2016
PLA	Usnic acid (UA)	NP's-PLA NP's-PLA-UA	Ø ≈246 nm PDI ≈ 0.1 ζ ≈ −25 mV spherical or slightly oval	NP's-PLA-UA reduced the cell viability in 70% over B16F10 melanoma cell line.	Antonio *et al.*, 2016
PEG-b-PDTC	Doxorubicin (DOX)	SCID-Ms cRGD/SCID-Ms DOX-SCID-Ms cRGD/DOX-SCID-Ms DOX-LPs	Ø ≈ 150 nm PDI ≈ 0.18 ζ- Neutral	DOX-SCID-Ms increased mice survival and decreased systemic toxicity in B16 melanoma tumor model *in vivo* compared to free DOX; cRGD20/DOX-SCID-Ms exhibited better therapeutic efficacy and lower side effects than DOX-LPs *in vivo*.	Zou *et al.*, 2016
PCL-PEI and PCL-PEG	Hedgehog pathway inhibitor vismodegib (VIS) and microRNA-34a (34a)	VIS/PHM/34a	Ø ≈ 60 nm PDI ≈ 0.3 ζ= +4.3 mV spheres	VIS/PHM/34a showed a synergistic anticancer efficacy in B16F10-CD44+ metastatic melanoma model.	Li *et al.*, 2015
PEG-CMC	Docetaxel (DTX)	DTX-PEGylated-CMC	Ø ≈118 nm PDI ≈ 0.1 ζ ≈ −22 mV	DTX-PEGylated-CMC increasing the tumor accumulation compared to Abraxane® in B16F10 melanoma model.	Ernsting *et al.*, 2012

PEG-PLA	Hydrophobic porphyrin derivative (Por)	Por-PEG-PLA	Ø ≈ 80 nm ζ ≈ −7.3 mV	Por-PEG-PLA showed *in vitro* phototoxicity in B16BL6 melanoma cells.	Ogawara *et al.*, 2016
PLGA	PI3K inhibitor (LY)	LY-PLGA	Ø≈ 110 nm spheres	NP-LY demonstrated a higher antiangiogenic response compared to free LY in B16F10 melanoma zebrafish xenograft model.	Harfouche *et al.*, 2009
mPEG-b-p(HPMAm-Lacn)	DOX	DOX-polymeric micelles	Ø ≈80 nm spheres	pH responsive DOX micelles prolonged survival compared to free DOX group with no adverse effects in B16F10 melanoma tumor model.	Talelli *et al.*, 2010
LMWH	DOX	LH-DOX-NPs	Ø ≈155 nm PDI≈ 0.2 ζ ≈ −35 mV spheres	pH responsive LH-DOX significantly reduced the tumor growth in B16F10 melanoma tumor model.	Mei *et al.*, 2016
Aldehyde-PEG-PLA and MPEG-PLA	DTX	TH10-DTX-NPs DTX-NPs	Ø ≈170 nm PDI≈ 0.2 ζ ≈ −22 mV spheres	TH10-DTX-NPs selective targeting the tumor vascular pericytes; promoted an increase in mice survival and low toxicity in B16F10-luc-G5 lung metastasis model *in vivo*.	Guan *et al.*, 2014
Albumin	Paclitaxel (PTX)	*nab*-PTX (Abraxane®) anti-VEGF conjugated with Abraxane®	Ø ≈160 nm	anti-VEGF conjugated with Abraxane® enhanced tumor regression compared to Abraxane® in human A375 melanoma model.	Nevala *et al.*, 2016

mPEG-b-PAGE	Epirubicin (EPI)	M(cbm), M(hz), cRGD-M(cbm) and cRGD-M(hz) conjugated with EPI	Ø ≈ 18, 50, 23, and 60 nm, respectively; spheres	pH sensitive cRGD-M presented a significant increasing anti-tumor activity in B16F10 melanoma xenograft model compared with free epirubicin.	Guan *et al.*, 2014
HACE	DOX	DOX-loaded HACE-based NPs	Ø ≈110 nm PDI≈ 0.2 ζ ≈ −24.3 mV spheres	Tumor growth was significantly inhibited by DOX-loaded NPs in the B16F10 melanoma model.	Jin *et al.*, 2012
Lactoferrin(Lf)	Fluorouracil (5-FU)	5-FU-LfNPs	Ø ≈150 nm PDI≈ 0.3 ζ ≈ −2.5 mV spheres	pH responsive 5-FU-LfNPs prolonged intracellular retention and enhanced cytotoxicity effect (2.7 fold) compared to the free 5-FU in B16F10 cells.	Kumari & Kondapi, 2016
PLGA, PEG-b-PLGA and PEG-b-PCL	antigens (Melan-A:26, gp100:209 or gp100:44) TLR ligands: Poly(I:C) and CpG	Man-NPs	Ø ≈145 to 190 nm PDI≈ 0.1 to 0.3 ζ ≈ −0.8 to −6.2 mV	Synergistic effect of immunopotentiators and potentiation of the anti-tumor immune response in B16F10 melanoma tumor model.	Silva *et al.*, 2015

However, experimental studies of nanoparticles designed to specifically target the m-CSCs and CMCs are still sparse and incipient. The recent literature involving polymer-based nanoparticles for melanoma diagnosis and treatment will be reviewed with focus on passive and active targeting to melanoma cells. We also analyzed the fundamentals and challenges behind the development of polymeric nanoparticles to target the m-CSCs/CMC, according with new insights about their biological mechanisms and biomarkers.

PASSIVE TUMOR-TARGETING OF DRUG LOADED NANOPARTICLES

In the early stages of tumor progression, it is demonstrated that solid tumors cannot grow further than 2 mm in diameters without angiogenesis (Folkman, 1971). To support the tumor growth, a high oxygen and nutrients are demanded, and these factors stimulate the uncontrolled angiogenesis. That phenomenon leads to leaky and intricate blood vessels, that are constantly under inflammatory state and it is associated with metastasis, tumor recurrence and poor survival rates (Banerjee *et al*., 2011; Bertrand *et al*., 2014). First described by Matsumura and colleges (1987), enhanced permeability and retention (EPR) phenomenon is based on these anatomical and pathophysiological properties of tumor microenvironment which can promote an accumulation of large molecules, such as proteins, through leaky vasculature and poor lymphatic drainage in the tumor (Bazak *et al*., 2014). Passive targeting of drugs is based on non-specific accumulation of drug-loaded nanoparticles in the tumor site, as a consequence of EPR effect (Danhier *et al*., 2010).

Structurally simple polymeric nanoparticles based on polyesters, as PCL and PLA, or cellulose polymers, such as carboxymethylcellulose, have been recently developed to improve the passive drug accumulation by EPR effect in melanoma tumor niche. Nanoparticles consisted of PEGylated carboxymethylcellulose conjugate with docetaxel (DTX) improved the specificity of delivery increasing 203-fold the tumor accumulation compared to the FDA approved Abraxane® in B16F10 melanoma models (Ernsting *et al*., 2012). Lipid-core nanocapsules, composed of PCL shell and caprylic triglyceride oil core, encapsulating acetyleugenol (AcE-LNC), were administered orally in B16F10 mice melanoma model. The treatment with empty LNC induced a higher reduction in the tumor volume when compared to the AcE-LNC and free AcE at the same dose. The authors explained these interesting results by the possible interactions between AcE and PCL altering the crystallinity of the polymer and the LNC supramolecular structure, decreasing the anti-tumor activity of AcE-LNC. These results imply the relevance of nanocapsule supramolecular structure to improve the passive targeting and cancer cells endocytosis, increasing the anti-melanoma therapeutic effect (Drewes *et al*., 2016). In another study, PLA nanoparticles containing ursolic acid (UA) were able to maintain the drug anti-melanoma activity in B16F10 cells, reducing the cell viability in 70%, and decrease the drug toxicity effects over normal cells (Antônio *et al*., 2017).

Another example of structurally simple and biocompatible polymeric nanocarriers are micellar nanoparticles, nanoscopic core/shell structures formed by amphiphilic block copolymers that can carrier both hydrophobic and hydrophilic drugs (Croy

& Kwon, 2006). Micellar nanoparticles were tested in B16 melanoma tumor models to enhanced drug accumulation by EPR effect. Self-crosslinkable and intracellularly de-crosslinkable micellar nanoparticles containing doxorubicin (DOX-SCID-Ms) showed low systemic toxicity and ability to suppress tumor growth and prolong survival in malignant B16 melanoma-bearing C57BL/6 mice, in a dose-dependent manner when compared to free DOX·HCl (Zou *et al*., 2016). On the other hand, polymeric hybrid micelles (PHMs) and their potential to co-deliver small anti-cancer molecules and nucleic acid has recently been reported. PHMs with different surface charges, varying from neutral to cationic, containing micro-RNA-34a (miR-34a), a well-defined tumor suppressor, and Hedgehog (Hh) pathway inhibitor vismodegib (VIS), were evaluated as target therapeutic agent for CSCs elimination. This study observed that neutral PHMs compared to cationic ones have the capability to overcome systemic biological barriers and improve the stability in blood circulation. Besides, the co-encapsulation of miR-34a and VIS into neutral PHMs showed a synergistic anti-cancer efficacy in *in vivo* B16F10-CD44^{+} melanoma model, presenting a higher tumor inhibition rate (80%) compared to PHM containing VIS (51.5%) or miR-34a (65%). These cells displayed CSC characteristics and tumorigenic ability compared to B16F10-CD44^{-} cells (Shi *et al*., 2014; Li *et al*., 2015).

Among the molecules that act as positive regulators of angiogenesis, the VEGF and TGF-β are the most investigated targets to anti-cancer therapy, in general (Otrock *et al*., 2007; Luo *et al*., 2016). Pittella and collegues (2012) demonstrated through the administration of VEGF siRNA in calcium phosphate/charge-conversional polymer hybrid nanoparticles *in vivo* that silencing of VEGF gene expression could importantly inhibit tumor growth up to 68% in subcutaneous pancreatic tumor models. In an advanced *in vivo* melanoma model, Xu and collegues (2014) demonstrated that a nanoparticle-delivered TGF-β can augment the efficacy of a vaccine based in lipid nanoparticles functionalized with mannose loaded with tumor antigens and inhibited tumor growth by 52% compared with vaccine treatment alone.

The use of PI3K inhibitors as antiangiogenic agents is has also been explored as a promising strategy to induce cancer cell apoptosis and inhibit cell proliferation. Harfouche and collegues (2009) reported that PLGA nanoparticles containing a selective PI3K inhibitor can inhibit both melanoma and breast cancer cells induced angiogenesis in zebrafish tumor xenograft model. These approaches provide promising platforms for anti-angiogenesis therapy and indirectly eradicate m-CSCs.

STIMULI RESPONSIVE NANOPARTICLES FOR MELANOMA TARGETING

A promising targeting strategy for theranostic approach to melanoma is the development of nanoparticles that can be activated by different external stimuli, such as magnetism, photo-irradiation and temperature, or internal stimuli from the tumor microenvironment, such as extracellular and endosomal pH (Navarro *et al*., 2013; Cyphert *et al*., 2017). The fast activity, poor lymphatic drainage and their inefficient blood irrigation, tumor growth is carried out through hypoxia, anaerobic metabolism and acidosis conditions (Alimoradi *et al*., 2016). Therefore, tumor microenvironment can be significantly acid, with pH values ranged from 6.0 to 7.0 compared with normal pH tissue of 7.4 (Danhier *et al*., 2010). Throw the spotlight of tumor pH, nanoparticles with pH-sensitive biomaterials are currently formulated for drug delivery therapy. Those stimuli-responsive nanoparticles after passive accumulation at tumor site by EPR effect can release the drug near or *in-situ* to the target by either the degradation of the nanoparticle itself or degradation of nanoparticle's shell. The drug can be loaded either by covalent bonds to the bio-sensitive material or encapsulated into the nanoparticle's core (Ding *et al*., 2013).

The development of pH-sensitive polymeric nanoparticles has been intensively studied in recent years, specially to improve DOX intracellular and nuclei delivery. An ideal nano-delivery system for DOX require a dual pH-sensitivity nanoparticle, firstly to overcome the extracellular barrier of pH gradients in tumor microenvironment and secondly to overcome the increased acidity in intracellular compartments, such as endosomes (pH~5.0) and subsequently release DOX from nanocarriers (Xiong *et al*., 2010). The design of new optimized pH-sensitive drug delivery system for DOX can be a promising strategy to surpass the m-CSCs multidrug resistance mechanisms since the major chemotherapy obstacle is the inefficient and unspecific cellular uptake. Talelli and collegues (2010) developed a DOX-loaded core-crosslinked polymeric micelles, composed by thermosensitive block copolymer covalently bounded to DOX. In the *in vitro* cytotoxicity assay in melanoma cells the DOX micelles were less effective than free DOX, probably due to slower uptake of the polymeric micelles. However, in mice bearing B16F10 melanoma model this polymeric nanocarrier showed a significant decrease in the tumor growth rate than free DOX. These results indicate a better tumor accumulation, through the EPR effect, of polymeric micelles instead free drug. In the same way, Du and collegues (2011) developed a dual pH-sensitive polymeric nanoparticle and reported an enhanced anti-cancer efficiency and intracellular delivery in *in vitro* model of SK-3rd drug-resistant CSCs. These pH-sensitive nanoparticles demonstrated a higher internalization rate and cytoplasmic distribution at pH 6.8 than at pH 7.4 and a higher release rate at pH 5 (75%) than at pH 6.8 (25.5%). In most

recent study, a pH-responsive polymeric nanoparticle based-amphiphilic copolymer of low molecular weight heparin conjugated with doxorubicin (LH-DOX) significantly increased tumor growth inhibition (1.5-fold) compared to free DOX-treated group in mice bearing a B16F10 tumors (Mei *et al*., 2016).

Another type of stimuli responsive multifunctional nanoparticles for theranostic of malignant melanoma are based on photothermal therapy (PTT) and photodynamic therapy (PDT). These methods to intercept and kill skin cancer cells are based on nanoparticle light-heat conversion ability or singlet oxygen generation using the near-infrared (NIR) as the light source (Lv *et al*., 2015). As demonstrated by Navarro and collegues (2013), gold nanoparticles functionalized with luminescent block copolymers, labeled with dibromobenzene based chromophore, are efficient nanocarries for fluorescent imaging and PDT. These nanoparticles increased the local concentration of photosensitizer molecules, improving photoinduced cell death in B16F10 melanoma cells. In another study, PTT using PEGylated gold nanorods and NIR showed a significant reduction in tumor volume of approximately 80% compared to the control (saline + NIR light) and increase animal survival in a mouse melanoma model when compared to control groups (Popp *et al*., 2014). Another theranostic nanoparticle, made by surface attachment of a new indocyanine green dye (IR820) to magnetic iron oxide nanoparticles coated with chitosan, showing an excellent magnetic resonance imaging (MRI) capability when compared to IR820 and functioned as a PDT against A375 melanoma cells with the increase of nanoparticles concentration (16µg/mL) (Hou *et al*., 2016). The PDT was also recently employed by Ogawara and collegues (2016). In this research, polymeric nanoparticles composed by poly (ethylene glycol) (PEG) and PLA block copolymer, encapsulating hydrophobic porphyrin derivative, showed a significant *in vitro* phototoxicity in B16BL6 melanoma cells.

ARCHITECTURAL PROPERTIES OF NANOPARTICLES IN PASSIVE TARGETING

In order to take benefit of tumor microenvironment and the EPR effect, certain characteristics of nanoparticles should be evaluated, specifically the size, surface charge, shape and stealth. As explained by Matsumura and collegues (1987), small molecules or particles are not influenced by EPR effect. Among their results, the authors described that small molecules under 30 kDa do not exhibit EPR effect (Maeda, 2012; Upponi *et al*., 2014). Therefore, macromolecules above 40 kDa or 10-200 nm in size tend to accumulate more effectively in the tumor site rather than small molecules of 3 to 12 kDa or 2 to 3 nm in size (Upponi *et al*., 2014; Zhong *et al*., 2014).

Nevertheless, recently researches also emphasizes that nanoparticles ranged between 10 to 40 nm present a better pharmacokinetic and immunological profile when

compared to larger nanoparticles, specially because they are larger enough to prevent quickly renal excretion and sufficiently small to allow the EPR effect and penetrate through the dense cellular extracellular matrix (Kunjachan *et al*., 2014; Hou *et al*., 2016).

The EPR-mediate passive effect of a ~ 10 nm prototypic polymeric nanocarrier based on poly(N-(2-hydroxypropyl) methacrylamide) (p-HPMA) were evaluated in highly and poorly leaky tumor models and also compared with Arg-Gly-Asp (RGD) and (Asn-Gly-Arg) NGR-mediated active targeting. Study findings lead to conclude that for the tested ~ 10 nm prototypic nanocarriers the passive targeting was significantly more effective than active tumor targeting utilizing integrin-ligand peptides in both mice bearing tumor models (Kunjachan *et al*., 2014).

Regarding to surface charge of nanoparticles to anti-cancer drug delivery, neutral nanocarriers could exhibit a better tumor accumulation and consequently a favorable *in vivo* behavior (Gabizon & Papahadjopoulos, 1992; Ogawara *et al*., 2016). Considering the factors described above, the modulation of nanoparticle's geometry can also enhance their tumor accumulation by passive targeting and consequently their applications as drug delivery (Ponchel & Cauchois, 2016). Van De Ven and collegues (2012) evaluated the tumor accumulation of silicon nanoparticles with different shapes and sizes, plateloid (600 x 200nm and 1000×400 nm) and cylindroid (1500×200 nm). They observed that larger plateloid nano-sized particles had the higher accumulation efficiency (5% of the dose per gram organ) in tumors in a melanoma mice model, probably because of the large surface area of the nanoparticle that favors their interaction and adherence to tumoral microvasculature.

Another critical parameter in nanoparticle's properties is their capacity to avoid immune elimination. The formation of protein corona or the recognition by the complement complex, lead to a rapid clearance by the kupffer cells in the liver and macrophages in the spleen as a part of the reticuloendothelial (RES) system, limiting the circulation half-life (Bazak *et al*., 2014; Upponi *et al*., 2014; Fornaguera *et al*., 2015). Thus, the longevity of nanoparticles in the blood circulation is a critical parameter for passive targeting. Grafted polymers on nanoparticle's surface can enhance this property and the most world-wide polymer used to this aim is the PEG. In addition, this polymer exhibit a steric stabilization effect by its protective hydrophilic layer once its exhibit in nanoparticle's surface (Veronese & Pasut, 2005; Romberg *et al*., 2008).

ACTIVE TUMOR-TARGETING OF DRUG LOADED NANOPARTICLES

The passive EPR-mediated targeting presents some drawbacks to nanotechnology applications specially related to the large differences between the tumors types and

the inter- and intra-individual variability of the pathophysiological states (Kunjachan *et al*., 2014). On the other hand, the active targeting is based on specific cancer cells molecules exclusively or overexpressed on the cell surface or subcellular compartments, as well as on the other cells of tumor microenvironment, such as the endothelial cells of microvasculature (Bazak *et al*., 2014). Generally, the targeting moieties most utilized to build site-specific nanoparticles for cancer treatment are the antibodies, antibodies fragments, aptamers, peptides, nucleic acid-based ligands, carbohydrates and small molecules as folic acid (Bertrand *et al*., 2014; Zhong *et al*., 2014). The ligand-modified tumor-targeted nanoparticles aims to increase the receptor-mediated endocytosis improving the specificity, retention and accumulation of drug nanocarriers into tumor site, leading to an increase in therapeutic efficacy and a decrease in off-target effects (Arranja *et al*., 2017).

ACTIVE TARGETING OF MELANOMA CANCER CELLS

Regarding cancer cells active targeting, a largely described and well explored molecule to target hyaluronan receptors (CD44), also overexpressed in m-CSC lineages, is the polysaccharide hyaluronic acid (HA). This polysaccharide, biocompatible and biodegradable, has a wide potential to be utilized to construct multifunctional nanoparticles to cancer diagnosis and therapy. DOX loaded HA-ceramide based nanoparticles were investigated for *in vitro* cellular uptake ability and antitumor effect into B16F10 tumor-bearing mouse model and demonstrated a receptor-mediated endocytosis and significant tumor growth inhibition (Jin *et al*., 2012).

Gene-specific therapeutic approach based on polymeric nanoparticle for delivery of siRNA, has been tested in clinical trials for advanced melanoma treatment. A cyclodextrin-based polymeric nanoparticles, displaying target transferrin protein on their surface, were evaluated in phase I clinical trial according to the specificity and capacity to improve the intracellular delivery of siRNA to melanoma tissue. This study observed a significant reduction in the expression of the enzyme ribonucleotide reductase, as well as a dose-dependent accumulation of targeted nanoparticles in melanoma tumors (Davis *et al*., 2010). Nevertheless, the same research group reported that after one-year treatment, severe adverse effects occurred and 21% of the patients discontinued the treatment. These adverse events were attributed to the instabilities in the nanoparticles formulation (Zuckerman & Davis, 2015). Recently, an innovative pH-responsive nanocarrier using a lactoferrin as a matrix for the preparation of nanoparticles containing 5-fluorouracil was evaluated in B16 melanoma cells. The intracellular delivery of fluorouracil demonstrated to be pH dependent and the *in vitro* tests showed a receptor-mediated endocytosis and consequently a higher cytotoxic activity related to free fluorouracil (Kumari & Kondapi, 2016).

Otherwise, the development of active targeting nanoparticulate vaccines for melanoma prophylactic and therapeutic purposes was demonstrated for Silva and collegues (2015). In this study, PLGA polymeric nanoparticles coated with mannose for co-delivery of melanoma-associated antigens (Mart-1 and gp100) and toll-like receptor ligands (immunopotentiators) were developed. These nanoparticles, tested *in vivo* in murine B16F10 melanoma tumors, demonstrated a synergistic effect of immunopotentiators to induce a long lasting Th1 immune response; and the combination of toll-like receptor ligands with melanoma antigens potentiate the anti-tumor immune response, activating both $CD4^+$ and $CD8^+$ T-cells in the efficacy of the anti-tumor immune response.

A novel drug-delivery strategy for active targeting of cancer cells is based on the rational design of immune cells, especially macrophages, to carry nanoparticles to tumors (Choi *et al*., 2012). Recently, Xie and coworkers (2017) developed a theranostic drug delivery system consisted of macrophages carrying photoluminescent polymeric nanoparticles loaded with anti-BRAF specific drug. The macrophages carrying the nanoparticles were able to effective deliver drugs to melanoma cells throught cell-cell specific active binding.

The active targeting of CTCs/CMCs by functionalized nanoparticles for diagnosis, treatment, and post-therapeutic follow-up, is until an under-exploited strategy and remains a great challenge in nanotechnology. The main challenges involved in CTCs/CMCs targeting by nanomedicines are the rarity of these cells in peripheral blood, their short circulation time and their heterogeneous subpopulation, that can present different phenotypes and could express epithelial (non-CSCs) or/and CSCs biomarkers (Li *et al*., 2015). Nevertheless, some alternatives can be used to surpass these drawbacks and intercept the CTCs/CMCs in blood flow, such as the design of multifunctional nanoparticles with different ligands to target both epithelial and mesenchymal biomarkers on the CMCs surface. However, until this present study, CellSearch® system is the only platform approved by FDA for CTCs screening/diagnostics. However, as this technique is based on EpCAM expression on the surface of cancer cells, their application to other EpCAM negative cancers, as melanoma, is limited. In view of methodologic limitations for CMCs detection in blood, Seenivasan and collegues (2015) developed an electrochemical immunosensing system composed by silica nanoparticles functionalized with MCR1 antibody. The detection limit of this nanocarrier was 20 cells/mL for melanoma cells in peripheral blood of patients. Most recent, Li and coworkers (2017), developed a colorimetric nanoplataform capable of successfully detect CMCs (13 cells/mL) by bifunctional magnetic nanoparticles, which combined magnetic separation properties with nanozymes. In this study, the CMCs were first detected and isolated by magnetic nanoparticles functionalized with mela-

noma-associated chondroitin sulfate proteoglycan and only the CMCs bound to magnetic nanoparticles presented a catalytic ability to generate a blue oxidation product for colorimetric evaluation.

The assessment of cancer signaling pathways to perform molecular tumor profile is also very important for appropriate treatment choices and post-therapy follow-up. The micro-nuclear magnetic resonance (μNMR) is one of the methods available for this approach. The CMCs expression of melanocyte biomarkers, such as MART-1, and MAP kinase signaling molecules were assessed by μNMR through an iron oxide nanoparticle conjugation with specific antibodies. The results of this research appointed a correlation between the CMCs biomarkers expression levels and metastatic burden (Gee *et al*., 2016).

ACTIVE TARGETING OF TUMOR ENDOTHELIUM

The synthetic peptide Arg–Gly–Asp (RGD) sequence is one of the most common targeting ligand used in nanoparticles for active targeting of tumor endothelium (Park *et al*., 2004; Singh *et al*., 2009; Choi *et al*., 2017). This target moiety can strongly bind to $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin receptors, generally overexpressed in different cancer types, such as prostate carcinoma, breast cancer and melanoma, as well as in endothelial cells of tumor vasculature, specially during tumor progression and metastasis (Contois *et al*., 2015; Guan *et al*., 2014a; Guan *et al*., 2014b; Amin *et al*., 2015; He *et al*., 2015). The vasculature tumor targeting has been employed as a promising approach to complement the EPR effect-mediated passive targeting, directly facilitating the nanoparticle internalization into tumor cells, after their extravasation through the microvasculature (Bertrand *et al*., 2014; Amin *et al*., 2015).

The development of active targeting nanoparticles to overcome chemotherapy resistance of melanoma cancers has been widely investigated in last decade. As previously discussed, the use of nanotechnology can improve the therapeutic efficacy of classic chemotherapeutic agents that have a limited clinical application due to their poor pharmacokinetic properties, high toxicity to normal cells and acquired drug resistance. In a study conducted in B16F10 melanoma cell line, pH sensitive and site-specific nanoparticles composed of RGD-linked copolymer, encapsulating epirubicin, demonstrated a pH sensitive drug controlled release and a selective cellular uptake. This nanoparticle presented a significant increased anti-tumor activity *in vivo* and a lower systemic toxicity compared with free drug (Guan *et al*., 2014c). Zou and collegues (2016) developed self crosslinkable and intracellularly decrosslinkable biodegradable micellar nanoparticles containing DOX (DOX-SCID-Ms) for passive targeting and active targeting. In active targeting purpose, they compared RGD-decorated DOX-SCID-Ms with pegylated liposomal doxorubicin (DOX-LPs). The *in vivo* results per-

formed in malignant B16 melanoma model demonstrated a 3-fold higher drug tumor accumulation, low systemic toxicity, and a markedly improved survival rate for RGD-decorated DOX-SCID-Ms.

Another example of ligand for tumor vasculature is the anti-VEGF (bevacizumab) that can be conjugated on nanoparticle's surface to promote active targeting. A phase II clinical assessment of nanoparticle albumin-bound paclitaxel (Nab-PTX) combinated with bevacizumab treatment, concluded that this combination therapy significantly improved the clinical efficacy of PTX and increased the progression-free survival rate and the overall survival rate of patients with unresectable metastatic melanoma (Spitler *et al*., 2015). Most recently, Nab-PTX non-covalently conjugated with bevacizumab significantly improve the biodistribution of paclitaxel into tumor tissue and enhanced tumor regression compared to Nab-PTX in *in vivo* human melanoma xenograft model (A375) (Nevala *et al*., 2016).

Despite of promising results of targeting tumor microvasculature through receptor-ligand interaction, care should be taken to nonspecific targeting drug delivery, once integrin and VEGF receptors are widespread in normal or inflamed tissues (Sun *et al*., 2015). Paradoxically, the use of RGD-based peptides can accelerate tumor progression in mouse B16F0 melanoma and in Lewis lung carcinoma tumor grafts by inducing endothelial migration (Reynolds *et al*., 2009). To overcome this problem, Redko and collegues (2016) recently developed non-RGD cyclic $\alpha_v\beta_3$ peptide conjugated with Camptothecin for targeted drug delivery and reported a specific and strong binding affinity both *in vitro* and *in vivo* in a xenograft human metastatic melanoma model, improving the anti-tumor activity and reducing the off-targeted toxicity.

Besides integrins and VEGF receptors, melanoma-associated chondroitin sulfate proteoglycan (NG2), also strongly expressed in tumor vascular pericytes, have been emerging as a new target for antiangiogenic therapy. The efficacy of nanoparticles for DTX delivery conjugated with TH10 peptide to target NG2 receptors in tumor vasculature were investigated in mice bearing B16F10-luc-G5 melanoma experimental lung metastasis. The NG2-binding peptide TH10 promoted a specific mediated endocytosis of nanoparticles in tumor pericytes and significantly increased the mice survival, with low toxicity related (Guan *et al*., 2014a).

ARCHITECTURAL PROPERTIES OF NANOPARTICLES IN ACTIVE TARGETING

The surface modification of nanoparticles through the conjugation of target ligands, such as peptides and antibodies, could directly affect the *in vivo* performance of these surface modified nanocarriers. The main nanoparticle characteristics that can

influence the targeted cancer chemotherapy are the size, shape, surface charge and ligands density (Bertrand *et al*., 2014).

The architectural properties of nanoparticles can determine their biodistribution, bioavailability, endocytosis pathway and diffusion mobility within the cytoplasm (Chou *et al*., 2011; Elsabahy & Wooley, 2012). Generally, according to the biological application of nanoparticle, the size could vary from 4 to 250 nm (Zhong *et al*., 2014). The varied sizes can strongly dictate the pharmacokinetics and pharmacological behavior of the nanoparticles. All biological barriers have an average pore size range that delimits the diffusion of macromolecules and nanocarriers. In the vasculature of the mammalians, for example, particles with size below 5 nm can across the healthy endothelium to extracellular space; in case of tumor vasculature, leakier than normal endothelium, the pore size can be until 200 nm. At a cellular level, the size of endosomes can range from 60 to 120 nm, depending on the pathophysiological conditions, the endocytosis mechanisms and the nanoparticle physicochemical characteristics (Elsabahy & Wooley, 2012). The size influence of copolymeric nanoparticles consisted of natural polysaccharide hyaluronan (target CD44 receptors) and poly(γ-benzyl-L-glutamate) (PBLG) in active targeting were studied in *in vivo* lung cancer models. Nanoparticles with two different sizes were tested and the 30 nm nanoparticles demonstrated a more efficiently cellular uptake and a preferential active targeting of $CD44^+$ tumors when compared with the 300 nm nanoparticles after intravenous administration (Jeannot *et al*., 2016).

Another important characteristic that must be considered during nanoparticle design is the shape. Modifications in this parameter can modulate the drug solubilization capacity, immunogenicity, blood circulation time, toxicity and cell uptake (Elsabahy & Wooley, 2012; Bertrand *et al*., 2014; Zhong *et al*., 2014). Gratton and collaborators (2008), studied the shape effect of nanoparticles upper to 100 nm on cellular internalization by using HeLa cells. In this study, the rod-like particles, with high aspect ratios (ARs= 3; diameter = 150 nm, height = 450 nm), presented a higher uptake and consequently a higher *in vitro* cytotoxicity compared to more symmetric nanoparticles, such as spheres (diameter = 200 nm, height = 200 nm), in nonphagocytic cells. In the same way, Huang and collaborators (2010) studied the influence of various shaped mesoporous silica nanoparticles with different aspect ratios (ARs 1, 2, 4) in cell uptake of melanoma cell lineage A375 and concluded that the more elongated particles had higher non-specific uptake and faster internalization rates.

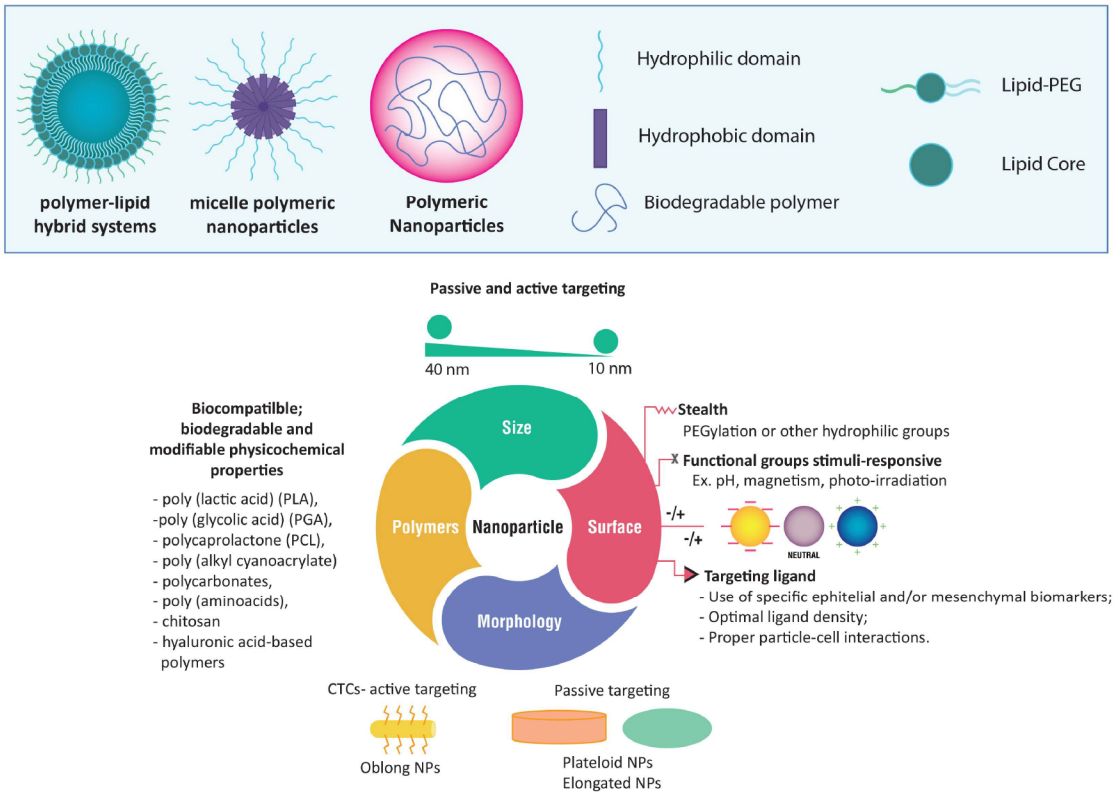
Regarding to CTCs/CMCs targeting, the nanoparticles shape can influence on their blood trafficking and the hemodynamic forces can affect the nanoparticle interactions with CTCs/CMCs and endothelial cells. It has been demonstrated that rod-like

nanoparticles could improve the margination onto the endothelium and the circulation time, potentiating the assessment of CTCs/CMCs into blood flow. These results can be explained probably due to larger surface areas of elongated nanoparticles which facilitates particle-cell and particle-vessel wall collisions and interactions. It is hypothesized that ligands coupled to the oblong nanoparticles can interact more efficiently with cell surface receptors, enhancing the number of specific bindings when compared with ligands attached to spherical particles, mainly due to the different surface areas in x and y axes (Albanese *et al*., 2012; Toy *et al*., 2014; Ponchel & Cauchois, 2016).

The number of ligands attached to the nanoparticle surface over a specific shape can directly affect their affinity and avidity by the bind target receptors at cell surface, as well as the membrane wrapping around the nanoparticle. The strength of ligand-coated nanoparticles and target cells interactions is analyzed as the avidity of the entire nanocarrier (Albanese *et al*., 2012). In general, an increase in ligands density on the surface of nanoparticles can lead to an increase in the target avidity and cellular internalization, nevertheless this correlation is not always linear and could have negative effects on cells interactions (Bertrand *et al*., 2014). Related to the nanoparticles surface charge, it has been demonstrated that higher positively particles tend to be more internalized when compared to more negatively ones in a non-specific way (Gratton *et al*., 2008; Albanese *et al*., 2012). This effect can be explained by the slightly negative charge of cells membrane which can attract by electrostatic force the positively nanoparticles, improving the cellular uptake. Nonetheless, an excess of positively charges is not recommended due to possible toxic and immunological effects (Elsabahy & Wooley, 2012). The surface charge can be optimized by changing nanoparticle materials and ligands density. For more reliable results, the effect of surface charge should be considering the tumor type and treatment arrangements, as well as the nanoparticles interactions with plasma and extracellular matrix. The plasma proteins could bind to nanoparticles surface and form a protein corona that can affect the particle-cell interactions (Monopoli *et al*., 2011).

In summary, it is essential to counterbalance the multiple physicochemical characteristics of nanoparticles, specially the size, shape, surface charge and ligands density, to improve the efficacy of these nanocarriers *in vivo*, according to the desired targets, for example, CMCs or m-CSCs. Besides, an ideal nanomedicine could also be designing to reach distinct types of tumorigenic cells at the same time using specific ligands that could target multiple cell types. Finally, according to the discussed studies, Figure 6 resumes the main used types of polymeric nanoparticles and the suitable physicochemical characteristics of these nanocarriers for targeting to CMC/m-CSC.

Figure 6 - Types of nanoparticles for metastatic melanoma treatment and suitable properties for the targeting of melanoma cells.



Source: Authors.

CHAPTER 7

CONCLUSIONS

The melanoma treatment with cytotoxic agents is difficult, specially due to the lack of drug specificity and multiple resistance mechanisms of cancer cells. Moreover, an ideal approach to prevent melanoma metastasis progression is the eradication of m-CSCs and CMCs presented in tumor site or in blood circulation. In this way, nanomedicines for specific recognition of these cells are still sparse and demand more attention from the scientific community. For targeting purpose, more accurately researches should be performed to detect and characterize more specific biomarkers present in m-CSCs and CMCs and to elucidate the signaling pathways involved in their maintenance and survival. Several studies point out that the polymeric nanoparticles are ideal platforms for the future tailoring and optimization of their surface physicochemical properties according to the pathophysiological peculiarities of each cancer. It is clear that architectural properties of nanoparticles can influence passive and active targeting of melanoma cells *in vitro* and *in vivo*. This book presented and discussed the current status of m-CSCs and CMCs biomarkers as potential targets for melanoma treatment using nanotechnological approaches. In conclusion, this book highlighted the challenging aspects of metastatic melanoma treatment and could guide future research to design polymeric nanoparticles that aiming to improve the clinical prognosis of this skin cancer.

REFERENCES

ABDULLAH, L. N.; CHOW, E. K.-H. Mechanisms of chemoresistance in cancer stem cells. **Clinical and translational medicine**, v. 2, n. 1, p. 1–9, 2013.

ABRUZZO, A. *et al.* Chitosan nanoparticles for lipophilic anticancer drug delivery: Development, characterization and in vitro studies on HT29 cancer cells. **Colloids and Surfaces B: Biointerfaces**, v. 145, p. 362–372, 2016.

ADAMS, D. L. *et al.* Cytometric characterization of Circulating Tumor Cells Captured by microfiltration and their correlation to the cellsearch?? CTC test. **Cytometry Part A**, v. 87, n. 2, p. 137–144, 2015.

AHRENS, T. *et al.* CD44 is the principal mediator of hyaluronic-acid-induced melanoma cell proliferation. **Journal of Investigative Dermatology**, v. 116, n. 1, p. 93–101, 2001.

ALBANESE, A. *et al.* [Review] The Effect of Nanoparticle Size, Shape, and Surface Chemistry on Biological Systems. **Annual Review of Biomedical Engineering**, v. 14, n. 1, p. 1–16, 2012.

ALBERTINI, M. R. *et al.* Phase IB trial of chimeric antidisialoganglioside antibody plus interleukin 2 for melanoma patients. **Clinical Cancer Research**, v. 3, n. August, p. 1277–1288, 1997.

ALIMORADI, H.; *et al.* Hypoxia Responsive Drug Delivery Systems in Tumor Therapy. **Current Pharmaceutical Design**, v. 22, n. 13, p. 2808–2820, 2016.

AMANN, V. C. *et al.* Developments in targeted therapy in melanoma. **European Journal of Surgical Oncology**, v. 43, n. 3, p. 581-593, 2016.

AMIN, M. *et al.* Development of a novel cyclic RGD peptide for multiple targeting approaches of liposomes to tumor region. **Journal of Controlled Release**, v. 220, p. 308–315, 2015.

ANTÔNIO, E. *et al.* Poly(lactic acid) nanoparticles loaded with ursolic acid: Characterization and in vitro evaluation of radical scavenging activity and cytotoxicity. **Materials Science and Engineering: C**, v. 71, p. 156–166, 2017.

ARRANJA, A. G. *et al.* Tumor-targeted nanomedicines for cancer theranostics. **Pharmacological Research**, v. 115, p. 87–95, 2017.

ASHWORTH, T. R. A case of cancer in which cells similar to those in the tumours were seen in the blood after death. Australian Medical Journal, v. 14.3, p. 146–149, 1869.

AURISICCHIO, L. *et al.* Superior Immunologic and therapeutic efficacy of a xenogeneic genetic cancer vaccine targeting carcinoembryonic human antigen. **Human Gene Therapy**, v. 398, n. June, p. 386–398, 2015.

AUST, L. *et al.* Yield of human adipose-derived adult stem cells from liposuction aspirates. **Cytotherapy**, v. 6, n. 1, p. 7–14, 2004.

BALCH, C. M. *et al.* Final version of 2009 AJCC melanoma staging and classification. **Journal of Clinical Oncology**, v. 27, n. 36, p. 6199–6206, 2009.

BANERJEE, D. *et al.* Nanotechnology-mediated targeting of tumor angiogenesis. **Vascular Cell**, v. 3, n. 1, p. 3, 2011.

BAO, G. *et al.* Multifunctional nanoparticles for drug delivery and molecular imaging. **Annual Review of Biomedical Engineering**, v. 15, n. April, p. 253–82, 2013.

BATTULA, V. L. *et al.* Ganglioside GD2 identifies breast cancer stem cells and promotes tumorigenesis. **The Journal of Clinical Investigation**, v. 122, n. 6, p. 2066–2078, 2012.

BAZAK, R. *et al.* Cancer active targeting by nanoparticles: a comprehensive review of literature. **Journal of Cancer Research and Clinical Oncology**, v. 141, n. Greish 2007, p. 769–784, 2014.

BENEZRA, M. *et al.* Multimodal silica nanoparticles are effective cancer-targeted probes in a model of human melanoma. **Journal of Clinical Investigation**, v. 121, n. 7, p. 2768–2780, 2011.

BERGMAN, P. J. *et al.* Development of a xenogeneic DNA vaccine program for canine malignant melanoma at the Animal Medical Center, **Vaccine**, v. 24, p. 4582–4585, 2006.

BERTRAND, N. *et al.* Cancer nanotechnology: The impact of passive and active targeting in the era of modern cancer biology. **Advanced Drug Delivery Reviews**, v. 66, p. 2–25, 2014.

BLUEMEL, C. *et al.* Epitope distance to the target cell membrane and antigen size determine the potency of T cell-mediated lysis by BiTE antibodies specific for a large melanoma surface antigen. **Cancer Immunology, Immunotherapy**, v. 59, p. 1197–1209, 2010.

BOMBELLI, F. B. *et al.* The scope of nanoparticle therapies for future metastatic melanoma treatment. **The Lancet Oncology**, v. 15, n. 1, p. 22–32, 2014.

BONNET, D. & DICK, J. E. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. **Nature medicine**, v. 3, n. 7, p. 730–737, 1997.

BOROVSKI, T. *et al.* Cancer stem cell niche: The place to be. **Cancer Research**, v. 71, n. 3, p. 634–639, 2011.

BORRULL, A. *et al.* Nanog and Oct4 overexpression increases motility and transmigration of melanoma cells. **Journal of Cancer Research and Clinical Oncology**, v. 138, n. 7, p. 1145–1154, 2012.

BRACHMANN, S. M. *et al.* Specific apoptosis induction by the dual PI3K/mTor inhibitor NVP-BEZ235 in HER2 amplified and PIK3CA mutant breast cancer cells. **Proceedings of the National Academy of Sciences of the United States of America**, v. 106, n. 52, p. 22299–22304, 2009.

BRIGGS, R. & KING, T. J. Transplantation of living nuclei from blastula cells into enucleated frogs' eggs. **Proceedings of the National Academy of Sciences**, v. 38.5, p. 455–463, 1952.

BROOKS, M. D.; BURNESS, M. L.; WICHA, M. S. Therapeutic implications of cellular heterogeneity and plasticity in breast cancer. **Cell Stem Cell**, v. 17, n. 3, p. 260–271, 2015.

BRUGGEN, P. V. D. *et al.* A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. **Science**, v. 254, n. 5038, p. 1643, 1991.

BRYS, A. K. *et al.* Nanotechnology-based strategies for combating toxicity and resistance in melanoma therapy. **Biotechnology Advances**, v. 34, n. 5, p. 565–577, 2016.

BU, Y. & CAO, D. The origin of cancer stem cells. **Frontiers in Bioscience**, v. S4, n. 2, p. 819–830, 2012.

BURG, M. A. *et al.* Expression of the NG2 proteoglycan enhances the growth and metastatic properties of melanoma cells. **Journal of Cellular Physiology**, v. 177, n. 2, p. 299–312, 1998.

BURKE, A. R. *et al.* Molecular Biomarkers & Diagnosis Targeting Cancer Stem Cells with Nanoparticle-Enabled Therapies. **Molecular Biomarkers & Diagnosis**, p. 8–11, 2012.

CALABRESE, C. *et al.* A Perivascular Niche for Brain Tumor Stem Cells. **Cancer Cell**, n. 11, p. 69–82, 2007.

CHEN, J. *et al.* Applications of nanotechnology for melanoma treatment, diagnosis, and theranostics. **International Journal of Nanomedicine**, v. 8, p. 2677–2688, 2013.

CHEN, K. G. *et al.* Principal expression of two mRNA isoforms (ABCB5a and ABCB5b) of the ATP-binding cassette transporter gene ABCB5 in melanoma cells and melanocytes. **Pigment Cell Research**, v. 18, n. 2, p. 102–112, 2005.

CHEN, M. *et al.* PLGA-nanoparticle mediated delivery of anti-OX40 monoclonal antibody enhances anti-tumor cytotoxic T cell responses. **Cellular Immunology**, v. 287, n. 2, p. 91–99, 2014.

CHEUNG, N.-K. V. *et al.* 3F8 monoclonal antibody treatment of patients with stage 4 neuroblastoma : a phase II study. **International Journal of Oncology**, v. 12, p. 1299–1306, 1998.

CHOI, B. S. *et al.* Phase I trial of combined treatment with ch14 . 18 and R24 monoclonal antibodies and interleukin-2 for patients with melanoma or sarcoma. **Cancer Immunology, Immunotherapy**, v. 55, n. 7, p. 761–774, 2006.

CHOI, J. *et al.* Targeting tumors with cyclic RGD-conjugated lipid nanoparticles loaded with an IR780 NIR dye: In vitro and in vivo evaluation. **International Journal of Pharmaceutics**, v. 532, n. 2, p. 677–685, 2017.

CHOU, L. Y. T. *et al.* Strategies for the intracellular delivery of nanoparticles. **Chemical Society Reviews**, v. 40, n. 1, p. 233–245, 2011.

CLEVERS, H. The cancer stem cell: premises, promises and challenges. **Nature Medicine**, v. 17, n. 3, p. 313–319, 2011

CONTOIS, L. W. *et al.* Inhibition of tumor-associated avb3 integrin regulates the angiogenic switch by enhancing expression of IGFBP-4 leading to reduced melanoma growth and angiogenesis in vivo. **Angiogenesis**, v. 18, n. 1, p. 31–46, 2015.

COUVREUR, P. & VAUTHIER, C. **Expert Review Nanotechnology : Intelligent Design to Treat Complex Disease**. [S.l.]: [s.n.], 2006.

COVAS, D. T. *et al.* Multipotent mesenchymal stromal cells obtained from diverse human tissues share functional properties and gene-expression profile with CD146+ perivascular cells and fibroblasts. **Experimental Hematology**, v. 36, n. 5, p. 642–654, 2008.

CROY, S. R. & KWON, G. S. Polymeric micelles for drug delivery. **Expert Opinion on Drug Delivery**, v. 3, n. 1, p. 139–162, 2006.

CSERMELY, P. *et al.* Cancer stem cells display extremely large evolvability: alternating plastic and rigid networks as a potential Mechanism: Network models, novel therapeutic target strategies, and the contributions of hypoxia, inflammation and cellular senescence. **Seminars in Cancer Biology**, v. 30, p. 1–10, 2014.

CYPHERT, E. L. *et al.* Chemotherapeutic delivery using pH-responsive, affinity-based release. **Experimental Biology and Medicine**, p. 1535370217693115, 2017.

DANHIER, F. *et al.* To exploit the tumor microenvironment: Passive and active tumor targeting of nanocarriers for anti-cancer drug delivery. **Journal of Controlled Release**, v. 148, n. 2, p. 135–146, 2010.

DAVEY, R. J. *et al.* Metastatic melanoma treatment: Combining old and new therapies. **Critical Reviews in Oncology/Hematology**, v. 98, p. 242–253, 2016.

DAVIS, M. E. *et al.* Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. **Nature**, v. 464, n. 7291, p. 1067–1070, 2010.

DEAN, M. ABC transporters, drug resistance, and cancer stem cells. **Journal of Mammary Gland Biology and Neoplasia**, v. 14, n. 1, p. 3–9, 2009.

DHULE, S. S. *et al.* Curcumin-loaded γ-cyclodextrin liposomal nanoparticles as delivery vehicles for osteosarcoma. **Nanomedicine : Nanotechnology, Biology, and Medicine**, v. 8, n. 4, p. 440–51, 2012.

DIETRICH, A. *et al.* Original Paper High CD44 Surface Expression on Primary Tumours of Malignant Melanoma Correlates with Increased Metastatic Risk and Reduced Survival. **European Journal of Cancer**, v. 33, n. 6, p. 926–930, 1997.

DING, H.-M. & MA, Y.-Q. Controlling Cellular Uptake of Nanoparticles with pH-Sensitive Polymers. **Scientific Reports**, v. 3, p. 2804, 2013.

DORONIN, I. I. *et al.* Ganglioside GD2 in reception and transduction of cell death signal in tumor cells. **BMC Cancer**, v. 14, p. 1–17, 2014.

DOU, J. *et al.* Isolation and identification of cancer stem-like cells from murine melanoma cell lines. **Cellular & Molecular Immunology**, v. 4, n. 6, p. 467–472, 2007.

DREWES, C. C. *et al.* Novel therapeutic mechanisms determine the effectiveness of lipid-core nanocapsules on melanoma models. **International Journal of Nanomedicine**, v. 11, p. 1261–1279, 2016.

DU, J. Z. *et al.* Tailor-Made dual pH-sensitive polymer-doxorubicin nanoparticles for efficient anticancer drug delivery. **Journal of the American Chemical Society**, v. 133, n. 44, p. 17560–17563, 2011.

DUAN, H. *et al.* Targeting endothelial CD146 attenuates neuroinflammation by limiting lymphocyte extravasation to the CNS. **Scientific reports**, v. 3, p. 1687, 2013.

EBOS, J. M. L. *et al.* Accelerated Metastasis after Short-Term Treatment with a Potent Inhibitor of Tumor Angiogenesis. **Cancer Cell**, v. 15, n. 3, p. 232–239, 2009.

_____ *et al.* Neoadjuvant antiangiogenic therapy reveals contrasts in primary and metastatic tumor efficacy. **EMBO molecular medicine**, 2014. v. 6, n. 12, p. 1561–76. Disponível em: <http://embomolmed.embopress.org/content/6/12/1561.abstract>.

ELSABAHY, M. & WOOLEY, K. L. Design of polymeric nanoparticles for biomedical delivery applications. **Chemical Society Reviews**, v. 41, n. 7, p. 2545–2561, 2012.

ELSHAL, M. *et al.* CD146 (Mel-CAM), an adhesion marker of endothelial cells , is a novel marker of lymphocyte. **Blood**, v. 106, n. 8, p. 2923–2924, 2005.

EROGLU, Z. & RIBAS, A. Combination therapy with BRAF and MEK inhibitors for melanoma: latest evidence and place in therapy. **Therapeutic Advances in Medical Oncology**, v. 8, n. 1, p. 48–56, 2016.

FANG, D. *et al.* A Tumorigenic Subpopulation with Stem Cell Properties in Melanomas. **Cancer Research**, p. 9328–9337, 2005.

FANG, J. *et al.* The EPR effect: Unique features of tumor blood vessels for drug delivery, factors involved, and limitations and augmentation of the effect. **Advanced Drug Delivery Reviews**, v. 63, n. 3, p. 136–151, 2011.

FARACE, F. *et al.* A direct comparison of CellSearch and ISET for circulating tumour-cell detection in patients with metastatic carcinomas. **British Journal of Cancer**, v. 105, p. 847–853, 2011.

FARGNOLI, M. C. *et al.* MC1R variants increase risk of melanomas harboring BRAF mutations. **The Journal of Investigative Dermatology**, v. 128, n. 10, p. 2485–2490, 2008.

FERRANDINA, G. *et al.* CD133 antigen expression in ovarian cancer. **BMC Cancer**, v. 9, p. 221, 2009.

FERRARI, M. Cancer nanotechnology: opportunities and challenges. **Nat Rev Cancer**, v. 5, n. 3, p. 161–71, 2005.

FLEIGE, E. *et al.* Stimuli-responsive polymeric nanocarriers for the controlled transport of active compounds: Concepts and applications. **Advanced Drug Delivery Reviews**, v. 64, n. 9, p. 866–884, 2012.

FOLKINS, C. *et al.* Anticancer therapies combining antiangiogenic and tumor cell cytotoxic effects reduce the tumor stem-like cell fraction in glioma xenograft tumors. **Cancer Research**, v. 67, n. 8, p. 3560–3564, 2007.

FOLKMAN, J. Tumor Angiogenesis: therapeutic implications. **The New England Journal of Medicine**, v. 18, n. Nov, p. 1182–1186, 1971.

FORNAGUERA, C. *et al.* Interactions of PLGA nanoparticles with blood components: protein adsorption, coagulation, activation of the complement system and hemolysis studies. **Nanoscale**, v. 7, n. 14, p. 6045–6058, 2015.

FREEMAN, J. B. *et al.* Evaluation of a multi-marker immunomagnetic enrichment assay for the quantification of circulating melanoma cells. **Journal of Translational Medicine**, v. 10, n. 1, p. 192, 2012.

FRIEDMANN-MORVINSKI, D. & VERMA, I. M. Dedifferentiation and reprogramming: Origins of cancer stem cells. **EMBO Reports**, v. 15, n. 3, p. 244–253, 2014.

GABIZON, A., & PAPAHADJOPOULOS, D. The role of surface charge and hydrophilic groups on liposome clearance in vivo. **Biochimica et Biophysica Acta (BBA)-Biomembranes**, v. 1103, n. 1, p. 94–100, 1992.

GACCHE, R. N. & MESHRAM, R. J. Angiogenic factors as potential drug target: Efficacy and limitations of anti-angiogenic therapy. **Biochimica et Biophysica Acta - Reviews on Cancer**, v. 1846, n. 1, p. 161–179, 2014.

GALLUZZI, L. *et al*. Doubling the blockade for melanoma immunotherapy. **OncoImmunology**, v. 5, n. 1, p. 1–4, 2015.

GAO, Y. *et al.* Nanotechnology-based intelligent drug design for cancer metastasis treatment. **Biotechnology Advances**, v. 32, p. 761–777, 2014.

GARCIA-MAZAS, C. *et al.* Biomaterials to suppress cancer stem cells and disrupt their tumoral niche. **International Journal of Pharmaceutics**, 2016.

GAY, L. *et al.*Tumour Cell Heterogeneity. **F1000Research**, v. 5, p. 238, 2016.

GEE, M. S. *et al.* Point of care assessment of melanoma tumor signaling and metastatic burden from µNMR analysis of tumor fine needle aspirates and peripheral blood. **Nanomedicine: Nanotechnology, Biology, and Medicine**, p. 1–8, 2016.

GHIORZO, P. *et al.* CDKN2A and MC1R analysis in amelanotic and pigmented melanoma. **Melanoma Research**, v. 19, n. 3, 2009.

GIBNEY, G. T. *et al.* Safety, correlative markers, and clinical results of adjuvant nivolumab in combination with vaccine in resected high-risk metastatic melanoma. **Clinical Cancer Research**, v. 21, n. 4, p. 712–720, 2015.

GRATTON, S. E. A. *et al.* The effect of particle design on cellular internalization pathways. **Proceedings of the National Academy of Sciences of the United States of America**, v. 105, n. 33, p. 11613–11618, 2008.

GRAY, E. S. *et al.* Circulating Melanoma Cell Subpopulations: Their Heterogeneity and Differential Responses to Treatment. **Journal of Investigative Dermatology**, v. 135, n. 8, 2015.

GRUNDY, M. *et al.* Advances in systemic delivery of anti-cancer agents for the treatment of metastatic cancer. **Expert Opinion on Drug Delivery**, v. 13, n. 7, p. 1–15, 2016.

GUAN, J. *et al.* Photodynamic action of methylene blue in osteosarcoma cells in vitro. **Photodiagnosis and Photodynamic Therapy**, v. 11, n. 1, p. 13–9, 2014.

GUAN, X. *et al.* Cyclic RGD targeting nanoparticles with pH sensitive polymer–drug conjugates for effective treatment of melanoma. **RSC Advances**, v. 4, n. 98, p. 55187–55194, 2014.

GUAN, Y. Y. *et al.* Selective eradication of tumor vascular pericytes by peptide-conjugated nanoparticles for antiangiogenic therapy of melanoma lung metastasis. **Biomaterials**, v. 35, n. 9, p. 3060–3070, 2014.

GUEZGUEZ, B. *et al.* Dual Role of Melanoma Cell Adhesion Molecule (MCAM)/CD146 in Lymphocyte Endothelium Interaction: MCAM/CD146 Promotes Rolling via Microvilli Induction in Lymphocyte and Is an Endothelial Adhesion Receptor. **The Journal of Immunology**, v. 179, p. 6673–6685, 2007.

GUO, R. *et al.* Micropthalmia Transcription Factor (MITF) as a diagnostic marker for metastatic melanomas negative for other melanoma markers. **International Journal of Clinical and Experimental Pathology**, v. 6, n. 8, p. 1658–1664, 2013.

GUPTA, P. B. *et al.* Cancer stem cells: mirage or reality? **Nature Medicine**, v. 15, n. 9, p. 1010–1012, 2009.

HAASE, O. *et al.* High Response Rate to Second-Line Combination Antiangiogenic Chemotherapy in Patients with Metastatic Melanoma. **Journal of Cancer Therapy**, v. 07, n. 12, p. 908–918, 2016.

HANDGRETINGER, R. *et al.* A Phase I Study of Human / Mouse Chimeric Anti- ganglioside GD2 Antibody ch14 . 18 in Patients with Neuroblastoma. **European Journal of Cancer**, v. 2, p. 261–267, 1995.

HAQUE, M. *et al.* Melanoma Immunotherapy in Mice Using Genetically Engineered Pluripotent Stem Cells. **Cell Transplantation**, v. 25, p. 811–827, 2016.

HARFOUCHE, R. *et al.* Nanoparticle-mediated targeting of phosphatidylinositol-3-kinase signaling inhibits angiogenesis. **Angiogenesis**, v. 12, n. 4, p. 325–338, 2009.

HARTMAN, M. L. *et al.* MITF in melanoma : mechanisms behind its expression and activity. **Cellular and Molecular Life Sciences**, p. 24–27, 2014.

HAYES, D. F. & PAOLETTI, C. Circulating tumour cells: Insights into tumour heterogeneity. **Journal of Internal Medicine**, v. 274, n. 2, p. 137–143, 2013.

HE, X. *et al.* RGD peptide-modified multifunctional dendrimer platform for drug encapsulation and targeted inhibition of cancer cells. **Colloids and Surfaces B: Biointerfaces**, v. 125, p. 82–89, 2015.

HENRY, N. L. & HAYES, D. F. Cancer biomarkers. **Molecular Oncology**, v. 6, n. 2, p. 140–146, 2012.

HERREROS-VILLANUEVA, M. *et al.* SOX2 promotes dedifferentiation and imparts stem cell-like features to pancreatic cancer cells. **Oncogenesis**, v. 2, n. 8, p. e61, 2013.

HERSEY, P. & ALESSANDROL, G. D. Expression of the gangliosides gm3 , gd3 and gd2 in tissue sections of normal skin , naevi , primary and metastatic melanoma. **International Journal of Cance**r, v. 343, p. 336–343, 1988.

HOU, S. *et al.* Polymer nanofiber-embedded microchips for detection , isolation , and molecular analysis of single circulating melanoma cells ** angewandte. **Angewandte Chemie (International ed. in English)**, v. 52, p. 3379–3383, 2013.

HOU, X. *et al.* Multifunctional near-infrared dye-magnetic nanoparticles for bioimaging and cancer therapy. **Cancer Letters**, v. 383, n. 2, p. 168–175, 2016.

HU, Z. *et al.* Human melanoma immunotherapy using tumor antigen-specific T cells generated in humanized mice. **Oncotarget**, v. 7, n. 6, p. 6448–6459, 2016.

HUANG, S. K. & HOON, D. S. B. Liquid biopsy utility for the surveillance of cutaneous malignant melanoma patients. **Molecular Oncology**, v. 10, n. 3, p. 450–463, 2016.

HUANG, S. *et al.* Tumor-targeting and smart nanoparticles for combination therapy of antiangiogenesis and apoptosis. **ACS NANO**, v. 7, n. 3, p. 2860-2871, 2013.

HUANG, X. *et al.* The effect of the shape of mesoporous silica nanoparticles on cellular uptake and cell function. **Biomaterials**, v. 31, n. 3, p. 438–448, 2010.

ISACKE, C. M. The role of the cytoplasmic domain in regulating CD44 function. **Journal of Cell Science**, v. 107, p. 2353–2359, 1994.

JANDL, T. *et al.* Melanoma stem cells in experimental melanoma are killed by radioimmunotherapy. **Nuclear Medicine and Biology**, v. 40, n. 2, p. 177–181, 2013.

JAYSON, G. C. *et al.* Antiangiogenic therapy in oncology: current status and future directions. **Lancet (London, England)**, v. 388, n. 10043, p. 518–529, 2016.

JIA, X. *et al.* Novel fluorescent pH/reduction dual stimuli-responsive polymeric nanoparticles for intracellular triggered anticancer drug release. **Chemical Engineering Journal**, v. 295, p. 468–476, 2016.

JIN, Y. J. *et al.* Hyaluronic acid derivative-based self-assembled nanoparticles for the treatment of melanoma. **Pharmaceutical Research**, v. 29, n. 12, p. 3443–3454, 2012.

JOPLING, C. *et al.* Dedifferentiation, transdifferentiation and reprogramming: three routes to regeneration. **Nature reviews. Molecular Cell Biology**, v. 12, n. 2, p. 79–89, 2011.

JOUR, G. *et al.* Angiogenesis in melanoma: an update with a focus on current targeted therapies. **Journal of Clinical Pathology**, p. 1–12, 2016.

KAKAVAND, H. *et al.* Targeted therapies and immune checkpoint inhibitors in the treatment of metastatic melanoma patients : a guide and update for pathologists. **Pathology**, v. 48, n. February, p. 194–202, 2016.

KAMALY, N. *et al.* Degradable Controlled-Release Polymers and Polymeric Nanoparticles: Mechanisms of Controlling Drug Release. **Chemical Reviews**, v. 116, n. 4, p. 2602–2663, 2016.

KELLY, P. N. *et al.* Tumor growth need not be driven by rare cancer stem cells. **Science**, v. 317, n. 5836, p. 337, 2007.

KENNEDY, C. *et al.* Melanocortin 1 receptor (MC1R) gene variants are associated with an increased risk for cutaneous melanoma which is largely independent of skin type and hair color. **Journal of Investigative Dermatology**, v. 117, n. 2, p. 294–30o, 2001.

KING, D. M. *et al.* Phase I clinical trial of the immunocytokine EMD 273063 in melanoma patients. **Journal of Clinical Oncology**, v. 22, n. 22, p. 4463–4473, 2004.

KOBAYASHI, N. C. C. & NORONHA, S. M. R. De. Cancer stem cells : a new approach to tumor development. **Revista da Associação Médica Brasileira**, v. 61, n. 1, p. 86–93, 2015.

KOCH, M. B. *et al.* Microphthalmia Transcription Factor and Melanoma Cell Adhesion Molecule Expression Distinguish Desmoplastic / Spindle Cell Melanoma From Morphologic Mimics. **The American Journal of Surgical Pathology**, v. 25, n. 1, p. 58–64, 2001.

KOLEV, V. N. *et al.* PI3K / mTOR Inhibitor VS - 5584 targets cancer stem cells and prevents tumor regrowth after chemotherapy in preclinical models of small cell lung cancer. **Cancer Research**, v. 12, n. 2, p. 33342, 2014.

KOWALCZYK, A. *et al.* The GD2-specific 14G2a monoclonal antibody induces apoptosis and enhances cytotoxicity of chemotherapeutic drugs in IMR-32 human neuroblastoma cells. **Cancer Letters**, v. 281, p. 171–182, 2009.

KREBS, M. G. *et al.* Circulating tumour cells: their utility in cancer management and predicting outcomes. **Therapeutic Advances in Medical Oncology**, v. 2, n. 6, p. 351–365, 2010.

KRUGER, G. M. *et al.* Neural crest stem cells persist in the adult gut but undergo changes in self-renewal , neuronal subtype potential , and factor responsiveness. **Neuron**, v. 35, p. 657–669, 2002.

KUMARI, S. & KONDAPI, A. K. Lactoferrin nanoparticle mediated targeted delivery of 5-fluorouracil for enhanced therapeutic efficacy. **International Journal of Biological Macromolecules**, v. 95, p. 232–237, 2016.

KUNJACHAN, S. *et al.* Passive versus active tumor targeting using RGD- and NGR--modified polymeric nanomedicines. **Nano Letters**, v. 14, n. 2, p. 972–981, 2014.

LEE, N. *et al.* Melanoma stem cells and metastasis: mimicking hematopoietic cell trafficking? **Laboratory Investigation; a Journal of Technical Methods and Pathology**, v. 94, n. 1, p. 13–30, 2014.

LEGHA, S. S. *et al.* Development of a biochemotherapy regimen with concurrent administration of cisplatin, vinblastine, dacarbazine, interferon alfa, and interleukin-2 for patients with metastatic melanoma. **Journal of Clinical Oncology**, v. 16, n. 5, p. 1752–1759, 1998.

LI, H. *et al.* Rational Design of Polymeric Hybrid Micelles with Highly Tunable Properties to Co-Deliver MicroRNA-34a and Vismodegib for Melanoma Therapy. **Advanced Functional Materials**, v. 25, n. 48, p. 7457–7469, 2015.

LI, J. *et al.* Nanobiotechnology for the Therapeutic Targeting of Cancer Cells in Blood. **Cellular and Molecular Bioengineering**, v. 8, n. 1, p. 137–150, 2015.

LI, J. *et al.* Recent advances in targeted nanoparticles drug delivery to melanoma. **Nanomedicine: Nanotechnology, Biology and Medicine**, v. 11, n. 3, p. 769–794, 2014.

LI, L. *et al.* Multifunctional "core-shell" nanoparticles-based gene delivery for treatment of aggressive melanoma. **Biomaterials**, v. 111, p. 124–137, 2016

LIANIDOU, E. S. *et al.* The Role of CTCs as Tumor Biomarkers. *In*: SCATENA, R. (Org.). **Advances in Cancer Biomarkers: From biochemistry to clinic for a critical revision**. Dordrecht: Springer Netherlands, p. 341–367, 2015.

LINK, C. *et al.* Factors Affecting the Clearance and Biodistribution of. **Molecular Pharmaceutics**, v. 5, n. 4, p. 505–515, 2008.

LIU, Y. *et al.* Multifunctional pH-sensitive polymeric nanoparticles for theranostics evaluated experimentally in cancer. **Nanoscale**, v. 6, n. 6, p. 3231–42, 2014.

LIU, Z. *et al.* Negative enrichment by immunomagnetic nanobeads for unbiased characterization of circulating tumor cells from peripheral blood of cancer patients. **Journal of Translational Medicine**, v. 9, n. 70, p. 1–8, 2011.

LOBO, N. A. *et al.* The biology of cancer stem cells. **Annual Review of Cell and Developmental Biology**, v. 23, p. 675–699, 2007.

LONGEE, D. C. *et al.* Disialoganglioside GD2 in human neuroectodermal tumor cell lines and gliomas. A**cta Neuropathologica**, v. 82, p. 45–54, 1991.

LÓPEZ, M. N. *et al.* Melanocortin 1 receptor is expressed by uveal malignant melanoma and can be considered a new target for diagnosis and immunotherapy. **Investigative Ophthalmology and Visual Science**, v. 48, n. 3, p. 1219–1227, 2007.

LUO, W. *et al.* Molecular cloning, expression analysis and miRNA prediction of vascular endothelial growth factor A (VEGFAa and VEGFAb) in pond loach Misgurnus anguillicaudatus, an air-breathing fish. **Comparative Biochemistry and Physiology Part - B: Biochemistry and Molecular Biology**, v. 202, p. 39–47, 2016.

LV, R. *et al.* Multifunctional anticancer platform for multimodal imaging and visible light driven photodynamic/photothermal therapy. **Chemistry of Materials**, v. 27, n. 5, p. 1751–1763, 2015.

MA, C. & ARMSTRONG, A. W. Severe adverse events from the treatment of advanced melanoma: a systematic review of severe side effects associated with ipilimumab, vemurafenib, interferon alfa-2b, dacarbazine and interleukin-2. **Journal of Dermatological Treatment**, v. 25, n. 5, p. 401–408, 2014.

MAIO, M. *et al.* Five-year survival rates for treatment-naive patients with advanced melanoma who received ipilimumab plus dacarbazine in a phase III trial. **Journal of Clinical Oncology**, v. 33, n. 10, p. 1191–1196, 2015.

MAK, A. B. *et al.* Post-translational regulation of CD133 by ATase1/ATase2-mediated lysine acetylation. **Journal of Molecular Biology**, v. 426, n. 11, p. 2175–2182, 2014.

MANCHUN, S. *et al.* Targeted therapy for cancer using pH-responsive nanocarrier systems. **Life Sciences**, v. 90, n. 11–12, p. 381–387, 2012.

MARCONI, A. *et al.* Hypoxia-Inducible Factor-1α and CD271 inversely correlate with melanoma invasiveness. **Experimental Dermatology**, v. 24, n. 5, p. 396–398, 2015.

MATSUMURA, Y. *et al.* General mechanism of intratumor accumulation of macromolecules: advantage of macromolecular therapeutics. **Cancer & Chemotherapy**, v. 14, n. (3 Pt 2), p. 821–829, 1987.

MAYNARD, A. D. Safe handling of nanotechnology. **Nature**, v. 444, n. 7117, p. 267, 2006.

MEACHAM, C. E. & MORRISON, S. J. Tumour heterogeneity and cancer cell plasticity. **Nature**, v. 501, n. 7467, p. 328–37, 2013.

MEI, L. *et al.* Polymer–Drug Nanoparticles Combine Doxorubicin Carrier and Heparin Bioactivity Functionalities for Primary and Metastatic Cancer Treatment. **Molecular Pharmaceutics**, p. 1–21, 2016.

MENG, F. *et al.* pH-sensitive polymeric nanoparticles for tumor-targeting doxorubicin delivery: concept and recent advances. **Nanomedicine (London, England)**, v. 9, p. 487–99, 2014.

MENG, Z. *et al.* Identification of an HLA-DPB1*0501 Restricted Melan-A/MART-1 Epitope Recognized by CD4+ T Lymphocytes: Prevalence for Immunotherapy in Asian Populations. **Journal of Immunotherapy**, v. 34, n. 7, p. 525–534, 2015.

MICHAELIS, M. *et al.* Enzastaurin inhibits ABCB1-mediated drug efflux independently of effects on protein kinase C signalling and the cellular p53 status. **Oncotarget**, v. 6, n. 19, p. 17605–17620, 2015.

MIETTINEN, M. *et al.* Microphthalmia transcription factor in the immunohistochemical diagnosis of metastatic melanoma: comparison with four other melanoma markers. **The American Journal of Surgical Pathology**, v. 25, n. 2, p. 205–211, 2001.

MOCKEY, M. *et al.* mRNA-based cancer vaccine: prevention of B16 melanoma progression and metastasis by systemic injection of MART1 mRNA histidylated lipopolyplexes. **Cancer Gene Therapy**, v. 14, n. 9, p. 802–814, 2007.

MONOPOLI, M. P. *et al.* Physical-Chemical aspects of protein corona: Relevance to in vitro and in vivo biological impacts of nanoparticles. **Journal of the American Chemical Society**, v. 133, n. 8, p. 2525–2534, 2011.

MONZANI, E. *et al.* Melanoma contains CD133 and ABCG2 positive cells with enhanced tumourigenic potential. **European Journal of Cancer**, v. 43, n. 5, p. 935–946, 2007.

MORATH, I. *et al.* CD44: More than a mere stem cell marker. **International Journal of Biochemistry and Cell Biology**, v. 81, p. 166–173, 2016.

MUKHERJEE, N. *et al.* Alternative Treatments For Melanoma: Targeting BCL-2 Family Members to De-Bulk and Kill Cancer Stem Cells. **The Journal of Investigative Dermatology**, v. 135, n. 9, p. 2155–2161, 2015.

MUKHERJEE, S. & RANJAN, C. Therapeutic application of anti angiogenic nanomaterials in cancers. **Nanoscale**, p. 12444–12470, 2016.

MURPHY, G. F. *et al.* Stem cells and targeted approaches to melanoma cure. Molecular Aspects of Medicine. **Molecular Aspects of Medicine,** v. 39, p. 33–49, 2014.

NAVARRO, J. R. G. *et al.* Nanocarriers with ultrahigh chromophore loading for fluorescence bio-imaging and photodynamic therapy. **Biomaterials**, v. 34, n. 33, p. 8344–8351, 2013.

NAVID, F. *et al.* Phase I Trial of a Novel Anti-GD2 Monoclonal Antibody , Hu14 . 18K322A , Designed to decrease toxicity in children with refractory or recurrent neuroblastoma. **Journal of Clinical Oncology**, v. 32, n. 14, p. 1445–1452, 2014.

NAZARIAN, R. M. *et al.* Melanoma biomarker expression in melanocytic tumor progression: A tissue microarray study. **Journal of Cutaneous Pathology**, v. 37, n. SUPPL. 1, p. 41–47, 2010.

NEGI, L. M. *et al.* Role of CD44 in tumour progression and strategies for targeting. **Journal of Drug Targeting**, v. 20, n. 7, p. 561–573, 2012.

NEVALA, W. K. *et al.* Antibody-targeted chemotherapy for the treatment of melanoma. **Cancer Research**, v. 76, n. 13, p. 3954–3964, 2016.

NIKOLAOU, V. *et al.* Antiangiogenic and antiapoptotic treatment in advanced melanoma. **Clinics in Dermatology**, v. 31, n. 3, p. 257–263, 2013.

NOTANI, K. *et al.* Amelanotic malignant melanomas of the oral mucosa. 2002. n. April 1982, p. 195–200.

OGAWARA, K. I. *et al.* Efficient anti-tumor effect of photodynamic treatment with polymeric nanoparticles composed of polyethylene glycol and polylactic acid block copolymer encapsulating hydrophobic porphyrin derivative. **European Journal of Pharmaceutical Sciences**, v. 82, p. 154–160, 2016.

ORDÓÑEZ, N. G. Value of melanocytic-associated immunohistochemical markers in the diagnosis of malignant melanoma: A review and update. **Human Pathology**, v. 45, n. 2, p. 191–205, 2014.

OTROCK, Z. K. *et al.* Understanding the biology of angiogenesis: Review of the most important molecular mechanisms. **Blood Cells, Molecules, and Diseases**, v. 39, n. 2, p. 212–220, 2007.

PÀEZ-RIBES, M. *et al.* Antiangiogenic Therapy Elicits Malignant Progression of Tumors to Increased Local Invasion and Distant Metastasis. **Cancer Cell**, v. 15, n. 3, p. 220–231, 2009.

PAGET, S. Distribution of secondary growths in cancer of the breast. **Lancet, I**, 1889. p. 571–573.

PALUNCIC, J. *et al.* Roads to melanoma: Key pathways and emerging players in melanoma progression and oncogenic signaling. **Biochimica et Biophysica Acta - Molecular Cell Research**, v. 1863, n. 4, p. 770–784, 2016.

PARHI, P. *et al.* Nanotechnology-based combinational drug delivery: An emerging approach for cancer therapy. **Drug Discovery Today**, v. 17, n. 17–18, p. 1044–1052, 2012.

PARK, J. H. *et al.* Self-assembled nanoparticles based on glycol chitosan bearing 5??-cholanic acid for RGD peptide delivery. **Journal of Controlled Release**, v. 95, n. 3, p. 579–588, 2004.

PIETILA, M. *et al.* Whom to blame for metastasis, the epithelial-mesenchymal transition or the tumor microenvironment? **Cancer Letters**, v. 380, n. 1, p. 359–368, 2016.

PIKTEL, E. *et al.* Recent insights in nanotechnology-based drugs and formulations designed for effective anti-cancer therapy. **Journal of nanobiotechnology**, v. 14, n. 1, p. 39, 2016.

PINC, A. *et al.* Targeting CD20 in Melanoma Patients at High Risk of Disease Recurrence. **Molecular Therapy**, v. 20, n. 5, p. 1056–1062, 2012.

PITTELLA, F. *et al.* Pancreatic cancer therapy by systemic administration of VEGF siRNA contained in calcium phosphate/charge-conversional polymer hybrid nanoparticles. **Journal of Controlled Release**, v. 161, n. 3, p. 868–874, 2012.

PONCHEL, G. & CAUCHOIS, O. Shape-Controlled Nanoparticles for Drug Delivery and Targeting Applications. **Polymer Nanoparticles for Nanomedicines.** [S.l.]: Springer International Publishing, 2016, p. 159–184.

POPP, M. K. *et al.* Photothermal therapy using gold nanorods and near- infrared light in a murine melanoma model increases survival and decreases tumor volume . **Journal of Nanomaterials**, v. 80301, 2014.

PORE, M. *et al.* Cancer stem cells, epithelial to mesenchymal markers, and circulating tumor cells in small cell lung cancer. **Clinical Lung Cancer**, v. 17, n. 6, p. 535–542, 2016.

PORTA, C. A M. & ZAPPERI, S. Human breast and melanoma cancer stem cells biomarkers. **Cancer Letters**, v. 338, n. 1, p. 69–73, 2013.

PRABHU, R. H. *et al.* Polymeric nanoparticles for targeted treatment in oncology: Current insights. **International Journal of Nanomedicine**, v. 10, p. 1001–1018, 2015.

PRIETO, V. G. & SHEA, C. R. Immunohistochemistry of Melanocytic Proliferations. **Archives of Pathology and Laboratory Medicine**, v. 135, p. 853–859, 2011.

PUCCHIO, T. Di *et al.* Immunization of Stage IV Melanoma Patients with Melan-A / MART-1 and gp100 Peptides plus IFN-α Results in the Activation of Specific CD8 + T Cells and Monocyte / Dendritic Cell Precursors. **Cancer Research**, v. 66, n. 9, 2006.

QUINTANA, E. *et al.* Phenotypic heterogeneity among tumorigenic melanoma cells from patients that is reversible and not hierarchically organized. **Cancer Cell**, v. 18, n. 5, p. 510–523, 2010.

RAMAKRISHNA, S. *et al.* Biomedical applications of polymer-composite materials: a review. **Composites Science and Technology**, v. 61, p. 1189–1224, 2001.

RAMPERSAUD, S. *et al.* The effect of cage shape on nanoparticle-based drug carriers: Anti-cancer drug release and efficacy via receptor blockade using dextran-coated iron oxide nanocages. **Nano Letters**, v. 16, n. 12, p. 7357–7363, 2016.

RAO, S. & PRESTIDGE, C. A. Polymer-lipid hybrid systems: merging the benefits of polymeric and lipid-based nanocarriers to improve oral drug delivery. **Expert Opinion on Drug Delivery**, v. 13, n. 5, p. 691–707, 2016.

RAPANOTTI, M. C. *et al.* Sequential molecular analysis of circulating MCAM / MUC18 expression : a promising disease biomarker related to clinical outcome in melanoma. **Archives of Dermatological Research,** v. 306, p. 527–537, 2014.

REDKO, B. *et al.* Toward the development of a novel non-RGD cyclic peptide drug conjugate for treatment of human metastatic melanoma. **Oncotarget**, v. 8, n. 1, p. 757-768, 2017.

REED, C. M. *et al.* Vaccination with melanoma helper peptides induces antibody responses associated with improved overall survival. **Clinical Cancer Research**, v. 21, n. 17, p. 3879–3887, 2015.

REYNOLDS, A. R. *et al.* Stimulation of tumor growth and angiogenesis by low concentrations of RGD-mimetic integrin inhibitors. **Nature Medicine**, v. 15, n. 4, p. 392–400, 2009.

RHEE, Y.-H. *et al.* Low-level laser therapy promoted aggressive proliferation and angiogenesis through decreasing of transforming growth factor-β1 and increasing of Akt/hypoxia inducible factor-1α in anaplastic thyroid cancer. **Photomed Laser Surg**, v. 34, n. 6, p. 229–35, 2016.

ROBERT, C. *et al.* Ipilimumab plus Dacarbazine for Previously Untreated Metastatic Melanoma. **The New England Journal o f Medicine Original**, v. 26, p. 2517–2526, 2011.

RODIC, S. *et al.*Detection methods of circulating tumor cells in cutaneous melanoma: A systematic review. **Critical Reviews in Oncology / Hematology**, v. 91, n. 1, p. 74–92, 2014.

ROMBERG, B. *et al.* Coatings for Long-Circulating Nanoparticles. **Pharmaceutical Research**, v. 25, n. 1, p. 55–71, 2008.

ROSENBERG, B. S. A. *et al.* Prospective Randomized Trial of the Treatment of Patients With Metastatic Melanoma Using Chemotherapy With Cisplatin, Dacarbazine, and Tamoxifen Alone or in Combination With Interleukin-2 and Interferon Alfa-2b. **Journal of Clinical Oncology**, v. 17, n. 3, p. 968–975, 1999.

ROSS, A. A. *et al.* Detection and viability of tumor cells in peripheral blood stem cell collections from breast cancer patients using immunocytochemical and clonogenic assay techniques. **Blood**, v. 82, n. 9, p. 2605–2610, 1993.

ROTH, M. *et al.* Ganglioside GD2 as a therapeutic target for antibody-mediated therapy in patients with osteosarcoma. **Cancer**, p. 548–554, 2014.

ROY, R. *et al.* Zinc oxide nanoparticles induce apoptosis by enhancement of autophagy via PI3K/Akt/mTOR inhibition. **Toxicology Letters**, v. 227, n. 1, p. 29–40, 2014.

RUSSELL, K. C. *et al.* Cell-surface expression of neuron-glial antigen 2 (NG2) and melanoma cell adhesion molecule (CD146) in heterogeneous cultures of marrow-derived mesenchymal stem cells. **Tissue engineering. Part A**, v. 19, n. 19–20, p. 2253–2266, 2013.

SAKARIASSEN, P. Ø. *et al.* Angiogenesis-independent tumor growth mediated by stem-like cancer cells. **Pnas**, v. 103, n. 44, p. 16466–16471, 2006.

SALEH, M. N. *et al.* Phase I Trial of the Murine Monoclonal Anti-G D2 Antibody 14G2a in Metastatic Melanoma. **Cancer Research**, v. 52, p. 4342–4347, 1992.

SALTARI, A. *et al.* CD271 downregulation promotes melanoma progression and invasion in 3- dimensional models and in zebrafish. **The Journal of Investigative Dermatology**, v. 136, n. 10, p. 2049–2058, 2016.

SANG, M. *et al.* MAGE-A family : Attractive targets for cancer immunotherapy. **Vaccine**, v. 29, n. 47, p. 8496–8500, 2011.

SARKAR, A. & SIL, P. C. Iron oxide nanoparticles mediated cytotoxicity via PI3K/AKT pathway: Role of quercetin. **Food and Chemical Toxicology**, v. 71, p. 106–115, 2014.

SATAPATHY, S. R. *et al.* Enhancement of cytotoxicity and inhibition of angiogenesis in oral cancer stem cells by a hybrid nanoparticle of bioactive quinacrine and silver : Implication of base excision repair cascade Enhancement of cytotoxicity and inhibition of angiogenesis in or. **Molecular Pharmaceutics**, p. 1–49, 2015.

SCATENA, R. *et al.* Circulating tumour cells and cancer stem cells: a role for proteomics in defining the interrelationships between function, phenotype and differentiation

with potential clinical applications. **Biochimica et Biophysica Acta**, v. 1835, n. 2, p. 129–43, 2013.

SCHATTON, T. *et al.* Identification of cells initiating human melanomas. **Nature**, v. 451, n. 7176, p. 345–349, 2008.

SCHEEL, C. & WEINBERG, R. A. Cancer stem cells and epithelial-mesenchymal transition: Concepts and molecular links. **Seminars in Cancer Biology**, v. 22, n. 5–6, p. 396–403, 2012.

SCHLAAK, M. *et al.* Regression of metastatic melanoma by targeting cancer stem cells ABSTRACT: **Oncotarget**, v. 3, n. 1, p. 22–30, 2012.

SCHMIDT, P. *et al.* Eradication of melanomas by targeted elimination of a minor subset of tumor cells. 2011.

SCHRAGE, A. *et al.* Murine CD146 is widely expressed on endothelial cells and is recognized by the monoclonal antibody ME-9F1. **Histochem Cell Biol**, v. 129, p. 441–451, 2008.

SEENIVASAN, R. *et al.* An electrochemical immunosensing method for detecting melanoma cells. **Biosensors and Bioelectronic**, p. 1–28, 2015.

SENSES, K. M. *et al.* Phenotype-based variation as a biomarker of sensitivity to molecularly targeted therapy in melanoma. **MedChemCommun.**, v. 8, p. 88–95, 2017.

SETHI, S. *et al.* Clinical advances in molecular biomarkers for cancer diagnosis and therapy. **International Journal of Molecular Sciences**, v. 14, n. 7, p. 14771–14784, 2013.

SHACKLETON, M. *et al.* Heterogeneity in cancer: cancer stem cells versus clonal evolution. **Cell**, v. 138, n. 5, p. 822–829, 2009.

SHAKHOVA, O. & SOMMER, L. Testing the cancer stem cell hypothesis in melanoma: The clinics will tell. **Cancer Letters**, v. 338, n. 1, p. 74–81, 2013.

SHARMA, S. *et al.* Trends in Analytical Chemistry PLGA-based nanoparticles : A new paradigm in biomedical applications. **Trends in Analytical Chemistry**, v. 80, p. 30–40, 2016.

SHI, J. *et al.* Cancer nanomedicine: progress, challenges and opportunities. **Nature Publishing Group**, p. 1–18, 2016.

SHI, S. *et al.* Systemic delivery of microRNA-34a for cancer stem cell therapy. **Angewandte Chemie - International Edition**, v. 52, n. 14, p. 3901–3905, 2013.

_____ *et al.* Dual drugs (microRNA-34a and paclitaxel)-loaded functional solid lipid nanoparticles for synergistic cancer cell suppression. **Journal of Controlled Release**, 2014. v. 194, p. 228–237. Disponível em: <http://dx.doi.org/10.1016/j.jconrel.2014.09.005>.

SHIOZAWA, Y. *et al.* Cancer stem cells and their role in metastasis. **Pharmacology & Therapeutics**, v. 138, n. 2, p. 285–93, 2013.

SHMELKOV, S. V. *et al.* CD133 expression is not restricted to metastatic colon cancer cells initiate tumors. **The Journal of Clinical Investigation**, v. 118, n. 6, p. 2111–2120, 2008.

SHUKLA, S. *et al.* ABC Transporters - 40 Years Part II. *In*: M., -Anthony (Org.). [S.l: s.n., s.d.], p. 227–272.

SIDDIQUI, I. A. *et al.* Excellent anti-proliferative and pro-apoptotic effects of (-)-epigallocatechin-3-gallate encapsulated in chitosan nanoparticles on human melanoma cell growth both in vitro and in vivo. **Nanomedicine : Nanotechnology, Biology, and Medicine**, v. 10, n. 8, p. 1619–1626, 2014.

SIEGLER, E. L. *et al.* Nanomedicine targeting the tumor microenvironment: therapeutic strategies to inhibit angiogenesis, remodel matrix, and modulate immune responses. **Journal of Cellular Immunotherapy**, v. 2, n. September, p. 1–10, 2016.

SIGALOTTI, L. *et al.* Cancer testis antigens in human melanoma stem cells: Expression, distribution, and methylation status. **Journal of Cellular Physiology**, v. 215, n. 2, p. 287–291, 2008.

SILVA, C. O. *et al.* Melanoma Prevention : Challenges and Progresses in Nanotechnology for Melanoma Prevention and Treatment. **CRC Concise Encyclopedia of Nanotechnology**, p. 453–470, 2016.

SILVA, J. M. *et al.* In vivo delivery of peptides and Toll-like receptor ligands by mannose-functionalized polymeric nanoparticles induces prophylactic and therapeutic anti-tumor immune responses in a melanoma model. **Journal of Controlled Release**, v. 198, p. 91–103, 2015.

SINGH, A. & SETTLEMAN, J. EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer. **Oncogene**, v. 29, n. 34, p. 4741–4751, 2010.

SINGH, S. R. *et al.* Intravenous transferrin, RGD peptide and dual-targeted nanoparticles enhance anti-VEGF intraceptor gene delivery to laser-induced CNV. **Gene Therapy**, v. 16, n. 5, p. 645–659, 2009.

SLOMINSKI, A. T. & CARLSON, J. A. Melanoma resistance: a bright future for academicians and a challenge for patient advocates. **Mayo Clinic Proceedings**, v. 89, n. 4, p. 429–433, 2015.

SMITH, F. R. *et al.* The Mutation p99 Asp-Tyr Stabilizes Y-A New , Composite Quaternary State of Human Hemoglobin. **Proteins Structure, Function, and Genetics**, v. 10, p. 81–91, 1991.

SPITLER, L. E. *et al.* Phase II Study of Nab -Paclitaxel and Bevacizumab as First-line Therapy for Patients with Unresectable. **American Journal of Clinical Oncology**, v. 38, n. 1, p. 61–67, 2015.

STAQUICINI, F. I. *et al.* A Subset of Host B Lymphocytes Controls Melanoma Metastasis through a Melanoma Cell Adhesion Molecule / MUC18-Dependent Interaction : Evidence from Mice and Humans. **Cancer Research**, v. 68, n. 20, p. 8419–8428, 2008.

SUN, L. *et al.* Strategies of polymeric nanoparticles for enhanced internalization in cancer therapy. **Colloids and Surfaces B: Biointerfaces**, v. 135, p. 56–72, 2015.

SUN, X.-X. & YU, Q. Intra-tumor heterogeneity of cancer cells and its implications for cancer treatment. **Acta pharmacologica Sinica**, v. 36, n. 10, p. 1219–27, 2015.

SWART, G. W. M. Activated leukocyte cell adhesion molecule (CD166/ALCAM): developmental and mechanistic aspects of cell clustering and cell migration. **European Journal of Cell Biology**, v. 81, n. 6, p. 313–321, 2002.

TALELLI, M. *et al.* Core-crosslinked polymeric micelles with controlled release of covalently entrapped doxorubicin. **Biomaterials**, v. 31, n. 30, p. 7797–7804, 2010.

TANNISHTHA, R. *et al.* Stem cells, cancer, and cancer stem cells. **Nature**, v. 414, n. November, p. 105–111, 2011.

TANNOCK, I. F., & ROTIN, D. Acid pH in tumors and its potential for therapeutic exploitation. **Cancer Research**, v. 49, n. 16, p. 4373–4384, 1989.

TAZZARI, M. *et al.* Melan-A/MART-1 immunity in a EWS-ATF1 translocated clear cell sarcoma patient treated with sunitinib: a case report. **BMC Cancer**, v. 15, n. 1, p. 1–8, 2015.

TCHOGHANDJIAN, A. *et al.* A2B5 cells from human glioblastoma have cancer stem cell properties. **Brain Pathology**, v. 20, n. 1, p. 211–221, 2010.

THAPA, R. & WILSON, G. D. The Importance of CD44 as a Stem Cell Biomarker and Therapeutic Target in Cancer. **Stem Cells International**, v. 2016, p. 1–15, 2016.

TOY, R. *et al.* Shaping cancer nanomedicine: the effect of particle shape on the in vivo journey of nanoparticles. **Nanomedicine (London, England)**, v. 9, n. 1, p. 121–34, 2014.

TRAN, K. A. *et al.* MeK inhibitors and their potential in the treatment of advanced melanoma: the advantages of combination therapy. **Drug Design, Development and Therapy**, v. 10, p. 43–52, 2016.

TU, Y.-T. *et al.* Expression of endothelial nitric oxide synthase and vascular endothelial growth factor in human malignant melanoma and their relation to angiogenesis. **Clinical and experimental dermatology**, v. 31, p. 413–418, 2006.

UPPONI, J. R. *et al.* Passive vs. Active Targeting: An Update of the EPR Role in Drug Delivery to Tumors. *In*: ALONSO, M. J.; GARCIA-FUENTES, M. (Org.). **Nano-Oncologicals: New Targeting and Delivery Approaches**. Springer I ed. [S.l.]: [s.n.], 2014, p. 3–45.

VAIDHYANATHAN, S. *et al.* **Factors Influencing the Central Nervous System Distribution of a Novel Phosphoinositide 3-Kinase/Mammalian Target of Rapamycin Inhibitor GSK2126458: Implications for Overcoming Resistance with Combination Therapy for Melanoma Brain Metastases s**. [S.l.]: [s.n.], 2016. v. 356.

VALYI-NAGY, K. *et al.* Stem cell marker CD271 is expressed by vasculogenic mimicry- forming uveal melanoma cells in three-dimensional cultures. 2012. n. February, p. 588–592.

VANSTEENKISTE, J. F. *et al.* Efficacy of the MAGE-A3 cancer immunotherapeutic as adjuvant therapy in patients with resected MAGE-A3-positive non-small-cell lung cancer (MAGRIT): a randomised, double-blind, placebo-controlled, phase 3 trial. **The Lancet Oncology**, v. 17, n. 6, p. 822–835, 2016.

VAUTHIER, C. & PONCHEL, G. Polymer Nanoparticles for In Vivo Applications: Progress on Preparation Methods and Future Challenges. **Polymer Nanoparticles for Nanomedicines**. [S.l.]: [s.n.], 2016, p. 3–16.

VEN, A. L. VAN DE *et al.* Rapid tumoritropic accumulation of systemically injected plateloid particles and their biodistribution. **Journal of Controlled Release**, v. 158, n. 1, p. 148–155, 2012.

VERONESE, F. M. & PASUT, G. PEGylation, successful approach to drug delivery. **Drug Discovery Today**, v. 10, n. 21, p. 1451–1458, 2005.

VINOGRADOV, S. & WEI, X. Cancer stem cells and drug resistance: the potential of nanomedicine. **Nanomedicine (London, England)**, v. 7, n. 4, p. 597–615, 2012.

VISVADER, J. E. & LINDEMAN, G. J. Cancer stem cells: Current status and evolving complexities. **Cell Stem Cell**, v. 10, n. 6, p. 717–728, 2012.

WADAJKAR, A. S. *et al.* Multifunctional particles for melanoma-targeted drug delivery. **Acta Biomaterialia**, v. 8, n. 8, p. 2996–3004, 2012.

WANG, X. *et al.* Functional Characterization of an scFv-Fc Antibody that Immunotherapeutically Targets the Common Cancer Cell Surface Proteoglycan CSPG4. **Microenvironment and Immunology**, v. 71, n. 24, p. 7410–7422, 2011.

WEIDLE, U. H. *et al.* TCR-MHC / Peptide Interaction : Prospects for New Anti-tumoral Agents. **Cancer Genomics & Proteomics**, v. 278, n. 11, p. 267–277, 2014.

WELSCH, N. *et al.* Adsorption of proteins to functional polymeric nanoparticles. **Polymer**, v. 54, n. 12, p. 2835–2849, 2013.

WIDMER, D. S. *et al.* Melanoma' s next top model, it is in the air. **Experimental dermatology**, v. 24, p. 659–660, 2015.

WILSON, B. J. *et al.* ABCB5 maintains melanoma-initiating cells through a proinflammatory cytokine signaling circuit. **Cancer Research**, v. 74, n. 15, p. 4196–4207, 2014.

WOUTERS, J. *et al.* The Human Melanoma Side Population Displays Molecular and Functional Characteristics of Enriched Chemoresistance and Tumorigenesis. **PLoS ONE**, v. 8, n. 10, p. 1–16, 2013.

WU, C. *et al.* Colloids and Surfaces B : Biointerfaces Co-delivery of multiple drug resistance inhibitors by polymer / inorganic hybrid nanoparticles to effectively reverse cancer drug resistance. **Colloids and Surfaces B: Biointerfaces**, v. 149, p. 250–259, 2017.

WU, X. *et al.* Improved SERS-Active Nanoparticles with Various Shapes for CTC Detection without Enrichment Process with Supersensitivity and High Specificity. **ACS Applied Materials and Interfaces**, p. 1–34, 2016.

WU, Y. & WU, P. Y. CD133 as a Marker for Cancer Stem Cells: Progresses and Concerns. **Stem Cells and Development**, v. 18, n. 8, p. 1127–1134, 2009.

XIAO, Y. F. *et al.* microRNA detection in feces, sputum, pleural effusion and urine: Novel tools for cancer screening (Review). **Oncology Reports**, v. 30, n. 2, p. 535–544, 2013.

XIE, J. *et al.* Nanotechnology for the delivery of phytochemicals in cancer therapy. **Biotechnology Advances**, v. 34, n. 4, p. 343–353, 2015.

XIONG, X. B. *et al.* The therapeutic response to multifunctional polymeric nano-conjugates in the targeted cellular and subcellular delivery of doxorubicin. **Biomaterials**, v. 31, n. 4, p. 757–768, 2010.

XU, X. & ZHONG, J. F. Circulating tumor cells and melanoma progression. **The Journal of Investigative Dermatology**, v. 130, n. 10, p. 2349–2351, 2010.

XU, Z. *et al.* Multifunctional nanoparticles co-delivering Trp2 peptide and CpG adjuvant induce potent cytotoxic T-lymphocyte response against melanoma and its lung metastasis. **Journal of Controlled Release**, v. 172, n. 1, p. 259–265, 2013.

_____ *et al.* Nanoparticle-delivered transforming growth factor-β siRNA enhances vaccination against advanced melanoma by modifying tumor microenvironment. **ACS Nano**, 2014. v. 8, n. 4, p. 3636–3645.

YADAV, L. *et al.*Tumour angiogenesis and angiogenic inhibitors: a review. **Journal of Clinical and Diagnostic Research: JCDR**, v. 9, p. XE01, 2015.

YOUNG, H. E. *et al.* Human pluripotent and progenitor cells display cell surface cluster differentiation markers CD10, CD13, CD56, and MHC class-I. **Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine (New York, N.Y.)**, v. 221, n. 1, p. 63–71, 1999.

YU, A. L. *et al.* Anti-GD2 antibody with GM-CSF, interleukin-2, and isotretinoin for neuroblastoma. **The New England Journal of Medicine**, v. 363, n. 14, p. 1324–1334, 2010.

_____ *et al.* Alterations of Glycosphingolipids in Embryonic Stem Cell Differentiation and Development of Glycan-targeting Cancer Immunotherapy. **Stem Cells and Development**, 2016. n. Special Issues: Stem Cells and Gene Therapy, p. 1–58.

YU, R. K. *et al.* Structures , Biosynthesis , and Functions of Gangliosides-an Overview. **Journal of Oleo Science**,v. 60, n. 10, p. 537–544, 2011.

YUAN, J. *et al.* Immunologic responses to xenogeneic tyrosinase DNA vaccine administered by electroporation in patients with malignant melanoma. **Journal of Immunotherapy of Cancer**, v. 1, n. 20, p. 1–11, 2013.

_____ *et al.* Novel technologies and emerging biomarkers for personalized cancer immunotherapy. **Journal for Immunotherapy of Cancer**, 2016. v. 4, p. 3.

ZAND, S. *et al.* Heterogeneity of Metastatic Melanoma. **American Journal of Clinical Pathology**, v. 146, n. 3, p. 353–360, 2016.

ZHANG, D. *et al.* Antiangiogenic agents significantly improve survival in tumor-bearing mice by increasing tolerance to chemotherapy-induced toxicity. **Proceedings of the National Academy of Sciences of the United States of America**, v. 108, n. 10, p. 4117–4122, 2011.

ZHANG, J. *et al.* **Circulating Tumor Cell Isolation and Analysis**. 1. ed. [S.l.]: Elsevier Inc., 2016. v. 75.

ZHONG, Y. *et al.* Ligand-directed active tumor-targeting polymeric nanoparticles for cancer chemotherapy. **Biomacromolecules**, v. 15, n. 6, p. 1955–1969, 2014.

ZHONG, Y. *et al.* Cancer stem cells sustaining the growth of mouse melanoma are not rare. **Cancer Letters**, v. 292, n. 1, p. 17–23, 2010.

ZOU, Y. *et al.* Self-crosslinkable and intracellularly decrosslinkable biodegradable micellar nanoparticles: A robust, simple and multifunctional nanoplatform for high-efficiency targeted cancer chemotherapy. **Journal of Controlled Release**, 2016.

ZUCKERMAN, J. E. & DAVIS, M. E. Clinical experiences with systemically administered siRNA-based therapeutics in cancer. **Nature Reviews Drug Discovery**, v. 14, n. 12, p. 843–856, 2015.

ZUO, Z.-Q. *et al.* Promoting tumor penetration of nanoparticles for cancer stem cell therapy by TGF-β signaling pathway inhibition. **Biomaterials**, 2016. v. 82, p. 48–59, 2016.

AUTHORS' CURRICULUM

Sarah Brandão Palácio

Tenente Farmacêutica Industrial da Força Aérea Brasileira. Doutora pelo Programa em rede de Pós-graduação em Nanotecnologia Farmacêutica (PPGNANO) e Doutora em Inovação terapêutica pela Université Paris-Saclay. Mestre em Ciências Farmacêuticas pela Universidade Federal de Pernambuco (UFPE). Experiência nas áreas de indústria farmacêutica, farmácia de manipulação, tecnologia farmacêutica, nanotecnologia e caracterização físico-química de biopolímeros.

Industrial Pharmacist Lieutenant of Brazilian Air Force. PhD by the Graduate Program in Pharmaceutical Nanotechnology (PPGNANO) and PhD in Therapeutic Innovation by Université Paris-Saclay. Master in Pharmaceutical Sciences from the Federal University of Pernambuco (UFPE). Experience in the pharmaceutical industry, pharmaceutical technology, nanotechnology and physico-chemical characterization of biopolymers.

An Young Sarahi Taylor Castillo

Doutora em Inovação Terapêutica pela Université Paris-Saclay, França. Doutora em Ciência dos Materiais na área de Nanomedicina pela Universidade de Sonora, México. Graduação em Ciências Médicas Laboratoriais pela Universidade de Sonora, México. Experiência na área de síntese de polímeros multifuncionais, com ênfase em aplicações farmacêuticas e biomédicas, especialmente para o tratamento de células cancerígenas circulantes metastáticas.

PhD in Therapeutic Innovation from Université Paris-Saclay, France. PhD in Materials Science in the field of Nanomedicine from the University of Sonora, Mexico. Graduated in Medical Laboratory Sciences from the University of Sonora, Mexico. Expert in synthesis of multifunctional polymers, with emphasis on pharmaceutical and biomedical applications, especially for the treatment of circulating metastatic cancer cells.

Francisco Humberto Xavier Junior

Professor/Pesquisador da Universidade Federal da Paraíba (UFPB), Departamento de Ciências Farmacêuticas, Laboratório de Biotecnologia Farmacêutica. Pós-doutor em Medicina Translacional (UCL, School of Pharmacy, London- UK) e Nanotecnologia Farmacêutica (PPGNANO/UFPE). Doutor em Biotecnologia pela Universidade Federal do Rio Grande do Norte (UFRN) e Innovation thérapeutique - Université Paris-Sud (França) em regime de co-tutela (Capes-Cofecub). Mestre em Ciências da

Saúde pela UFRN. Experiência na área de Farmácia, com ênfase em produtos nanobiotecnológicos.

Professor/Researcher of the Federal University of Paraiba (UFPB), Pharmaceutical Sciences Department, Laboratory of Pharmaceutical Biotechnology. Post-doctorate in Translational Medicine (UCL, School of Pharmacy, London- UK) and Pharmaceutical Nanotechnology (PPGNANO/UFPE). PhD in Biotechnology from the Federal University of Rio Grande do Norte (UFRN) and Innovation thérapeutique at Université Paris-Sud (France) under co-tutelage (Capes-Cofecub). Master in Health Sciences at UFRN. Expert in the Pharmaceutical technology, with an emphasis on nanobiotechnological products.

Isabella Macário Ferro Cavalcanti

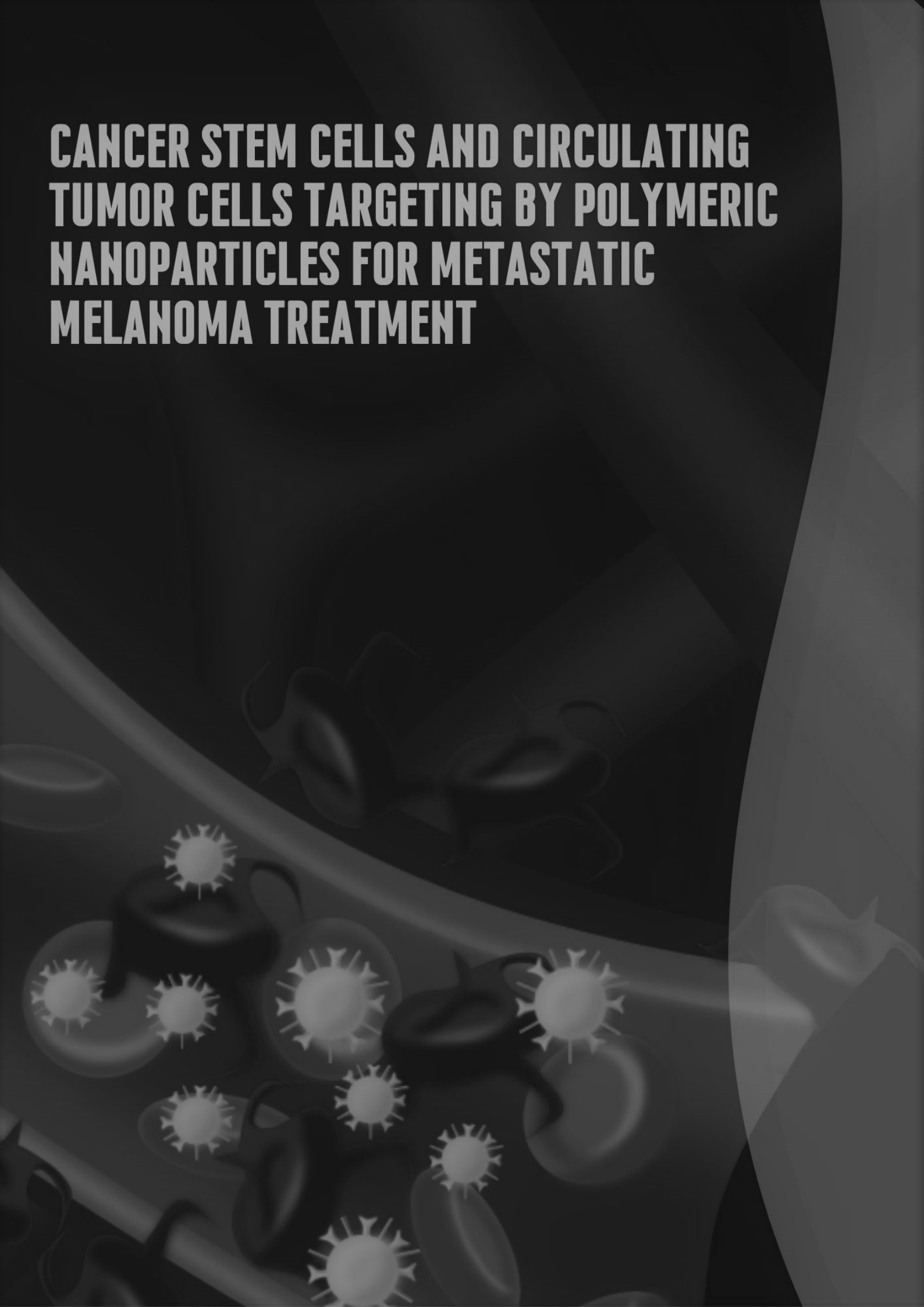
Professora Associada I das disciplinas de Microbiologia, Imunologia e Exames Laboratoriais no Centro Acadêmico de Vitória (CAV/UFPE). Membro do colegiado do Núcleo de Pesquisa e Extensão do CAV/UFPE (NPE/CAV/UFPE). Membro permanente do Programa de Pós-Graduação de Mestrado Profissional em Ensino de Biologia em Rede Nacional (PROFBIO), conceito 4 da CAPES. Chefe e Pesquisadora do Setor de Microbiologia Clínica do Laboratório de Imunopatologia Keizo Asami da Universidade Federal de Pernambuco (LIKA/UFPE). Membro da Sociedade Brasileira de Microbiologia. Membro do Comitê Científico e Consultivo de Apoio às Ações de Combate ao COVID-19 - CAV/UFPE. Líder do grupo de pesquisa Microbiologia clínica e Novas Abordagens Terapêuticas (MicroNAT). Doutora em Biotecnologia pelo Programa de Pós-graduação em Ciências Biológicas da UFPE (PPGCB/UFPE). Mestre em Inovação Terapêutica do Programa de Pós-graduação em Inovação Terapêutica da UFPE (PPGIT/UFPE). Especialista em Microbiologia Clínica pela Universidade de Pernambuco (UPE). Biomédica pela UFPE. Experiência na área de Nanotecnologia Farmacêutica e Microbiologia básica e aplicada.

Associate Professor I of the disciplines of Microbiology, Immunology and Laboratory Tests at the Academic Center of Vitória (CAV/UFPE). Member of the collegiate of the Research and Extension Nucleus of CAV/UFPE (NPE/CAV/UFPE). Permanent member of the Professional Master's Graduate Program in Biology Teaching in the National Network (PROFBIO), concept 4 of CAPES. Head and Researcher of the Clinical Microbiology Sector at the Laboratory of Immunopathology Keizo Asami at the Federal University of Pernambuco (LIKA/UFPE). Member of the Brazilian Society of Microbiology. Member of the Scientific and Advisory Committee to Support Actions to Combat COVID-19 - CAV/UFPE. Leader of the research group Clinical Microbiology and New Therapeutic Approaches (MicroNAT). PhD in Biotechnology by the Graduate Program in Biological Sciences at UFPE (PPGCB/UFPE). Master in Therapeutic

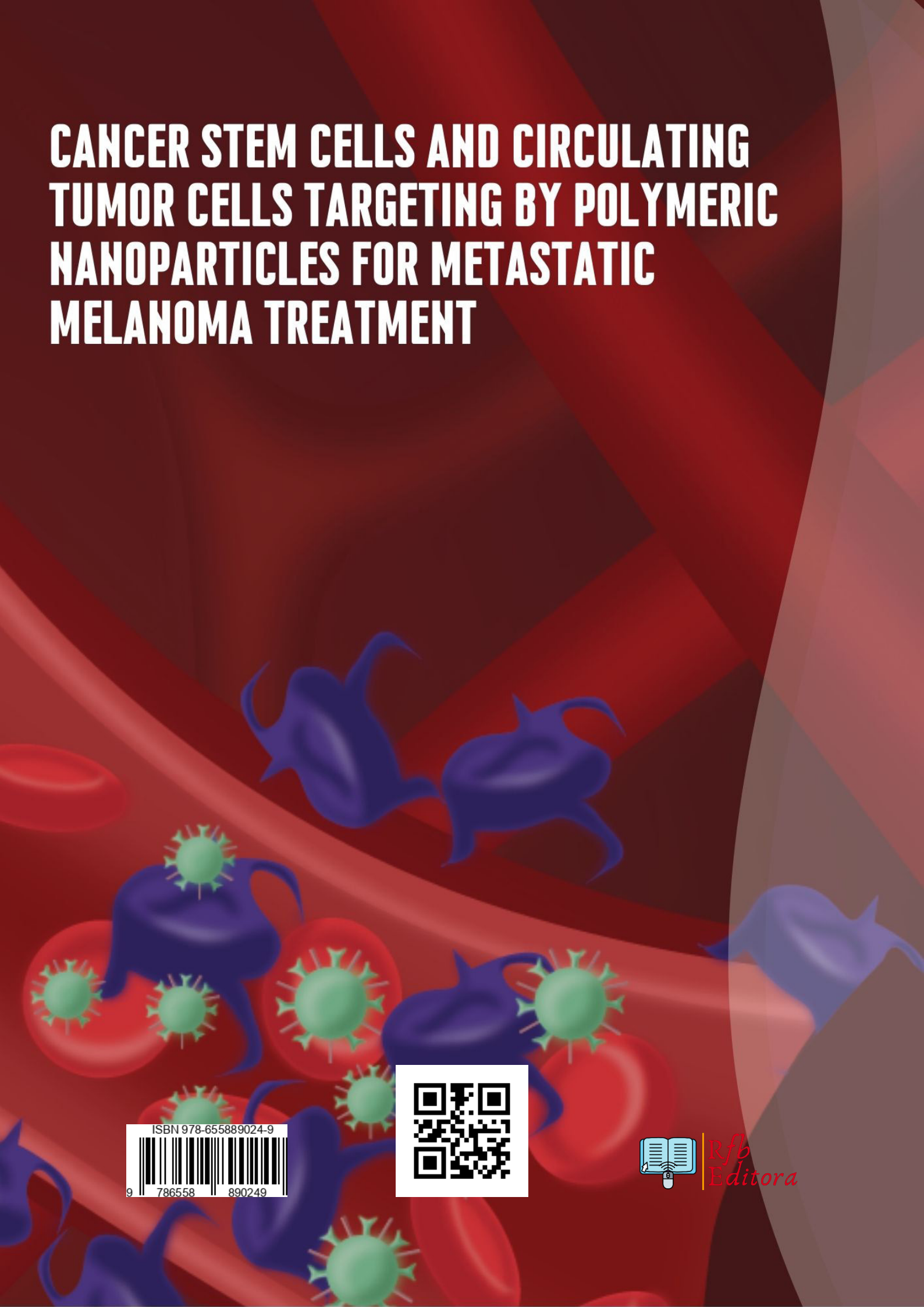
Innovation from the Postgraduate Program in Therapeutic Innovation at UFPE (PPGIT/UFPE). Specialist in Clinical Microbiology from the University of Pernambuco (UPE). Biomedical at UFPE. Expert in Pharmaceutical Nanotechnology and Basic and Applied Microbiology.

INDEX

CANCER STEM CELLS AND CIRCULATING TUMOR CELLS TARGETING BY POLYMERIC NANOPARTICLES FOR METASTATIC MELANOMA TREATMENT



CANCER STEM CELLS AND CIRCULATING TUMOR CELLS TARGETING BY POLYMERIC NANOPARTICLES FOR METASTATIC MELANOMA TREATMENT



ISBN 978-655889024-9

Rfb Editora