**This is a postprint of an article published in
Philippi, C., Loretz, B., Schaefer, U.F., Lehr, C.M.
Telomerase as an emerging target to fight cancer -
Opportunities and challenges for nanomedicine
(2010) Journal of Controlled Release, 146 (2), pp. 228-
240.**

Telomerase as an emerging target to fight cancer – opportunities and challenges for nanomedicine

C. Philippi, B. Loretz, U.F. Schaefer and C.M. Lehr*

Department of Biopharmaceutics and Pharmaceutical Technology, Saarland University, Saarbrücken
(GERMANY)

* to whom correspondence should be addressed.

e-mail: lehr@mx.uni-saarland.de

Phone: +49-681-302-3039

Fax: +49-681-302-4671

Key words:

telomerase inhibition, cancer therapy, G-quadruplex, carrier system, gene delivery, nanoparticles

Abstract

Telomerase as an enzyme is responsible for the renewal of the chromosomal ends, the so-called telomeres. By preventing them from shortening with each cell cycle, telomerase is able to inhibit cellular senescence and apoptosis. Telomerase activity, which is detectable in the majority of cancer cells, allows them to maintain their proliferative capacity. The thus obtained immortality of those cells again is a key to their malignancy.

Based on these discoveries, it is obvious that telomerase inhibitors would represent an innovative approach to fight cancer, and a variety of such candidate molecules are currently in the pipeline. Telomerase inhibitors largely fall in two classes of compounds: small synthetic molecules and nucleotide-based biologicals. For several candidates, some proof of concept studies have been demonstrated, either on cell cultures or in animal models. But the same studies also revealed that inefficient delivery is largely limiting the translational step into the clinic.

The most appealing feature of telomerase inhibitors, which distinguishes them from conventional anti-cancer drugs, is probably seen in their intrinsic non-toxicity to normal cells. Nevertheless, efficient delivery to the target cells, i.e. to the tumor, is still required. Here, some well-known biopharmaceutical problems such as insufficient solubility, permeability or even metabolic stability are frequently encountered. To address these challenges, there is a clear need for adequate delivery technologies, for example by using nanomedicines, that would allow to overcome their biopharmaceutical shortcomings and to warrant a sufficient bioavailability at the target side.

This review first briefly explains the concept of telomerase and telomerase inhibition in cancer therapy. It secondly aims to provide an overview of the different currently known telomerase inhibitors. Finally, the biopharmaceutical limitations of these molecules are discussed as well as the possibilities to overcome those limits by novel drug carrier systems and formulation approaches.

1. Introduction

1.1 Structure and function of telomerase

Telomerase is an enzyme that counteracts the molecular clock of eroding telomeres and thus acts as an immortalizing agent. Its principle of action and the cancer therapy approach emerging from that is depicted in Figure 1. In somatic cells telomeres get shortened in every mitosis and critically short telomeres act as trigger for apoptosis. In contrast in cancer cells the presence of active telomerase enzyme prevents telomere degradation and programmed cell death. A therapeutic anticancer approach, which inhibits telomerase function therefore, is capable of deprivation of unlimited progeny.

Telomerase is able to add new telomeric repeats to telomere ends [1, 2]. The enzyme consists of a catalytic protein unit, **human telomerase reverse transcriptase (hTERT)** and the **human telomerase RNA component (hTR)** which provides the template for the telomeric repeat sequence. The enzyme localizes at the 3' telomere end and adds successively new telomeric repeats by synthesis of DNA according to the RNA template. The elongation is processive. After a first addition of nucleotides to the 3' end of the telomere until the end of the template is reached, a translocation takes place and the elongation may continue for multiple cycles (see Fig. 2).

Telomerase function is highly regulated in normal cells. On the one hand via transcriptional regulation, alternative splicing and epigenetic regulation and on the other hand by the limited access to the chromosome ends [3-5]. Shelterin capping and G-quadruplexs stacks (shown in Fig. 3) influence the recruitment of telomerase to the telomere end [6]. Further the processivity of telomere elongation *in vivo* may be modulated by telomere or telomerase associated proteins.

Figure 1: Molecular basis for the therapeutical approach of cancer treatment by telomerase inhibition. In normal cells without telomerase activity the telomeres erode, while in cancer cells active telomerase maintain telomeres. The inhibition of telomerase activity deprives cancer cells of their function of unlimited progeny.

Figure 2: Telomerase enzyme cause telomere elongation by repeated addition of telomeric repeats to the 3' end of chromosomes. The subsequent lag strand synthesis this performed by a DNA polymerase.

1.2 Structure and function of telomeres

The linear arrangement of chromosomes necessitates structure elements to protect the ends from loss of information and from fusion with other chromosomes. These structures, called telomeres, are built by a repetitive DNA sequence and associated proteins [7]. Human telomere DNA sequences are repeats of the hexameric nucleotide sequence (TTAGGG) for some hundred times. These G-rich sequences are associated with certain specialised telomere binding proteins causing t-loop structures closing the ends [8].

The very end of telomere sequence is single stranded. This single stranded DNA is associated with two proteins POT1 and TPP1. The double stranded telomere sequence is associated with four telomere binding proteins: TRF1, TRF2, TIN 2 and RAP1. The capped conformation of telomeres is a structure where the single stranded DNA loops back into the double stranded part mediated by the interaction of the associated proteins. The whole protein complex is termed shelterin complex [9].

The second possible conformation for end protection is a formation of G-quadruplexes. These are planar stacks of G residues formed by intra- or intermolecular interaction of the G-rich single stranded telomere ends [10]. The two telomere end protective structures are pictured in Figure 3. Access to single stranded 3'-DNA overhangs at chromosomal ends is essential for telomere elongation by telomerase. Interfering with the t-loop formation or G-quadruplex stacks are methods to effect telomerase activity.

The telomere nucleoprotein structures fulfil a second important function in addition to maintenance of genomic stability. They act as a counter of cell divisions, supervising live time of cells [11]. Due to the mode of DNA replication, with the fragmented synthesis of the lag strand, chromosomes lose about 50 bp of their telomeric sequence in every mitosis. Reaching a critically short length leads to reduced association with the necessary proteins for the t-loop formation. Also G-quadruplex formation is minimized by short sequences. This critical length, known as “Hayflick limit” is reached after a certain number of cell divisions in normal somatic cells which then results in cell senescence or apoptosis [12].

Figure 3: Impact of open or capped telomere structure on the telomerase activity.

Free accessible 3' overhangs of G-rich sequence are necessary for telomere elongation by telomerase. Telomere associated proteins cap the chromosomal ends by formation of telomeric-loops. G-quadruplex formation between or within the G-tails also inhibit telomerase binding and function.

1.3 Telomerase as target for anticancer therapies

Telomerase activity is reported in 80-90% of tumors [13-17]. This is not surprising since one of the hallmarks of tumor is the indefinite growth managed by preventing telomere shortening and senescence. Several studies comprising a variety of mechanistic approaches like mutations, RNA interference, translational inhibition or functional inhibition of the telomerase enzyme activity provided the proof of concept for this form of cancer treatment [18-24]. Telomerase activity was even suggested as diagnostic cancer marker and prognosis factor [16, 25].

However, telomerase expression is also seen in a variety of normal tissues (blood cells, basal skin layer cells, epithelial cells and endothelial cells) at least during S-phase of the cell cycle [26]. Telomerase activity is mainly growth-regulated [27, 28]. In actively dividing cells a strictly regulated expression and telomerase activity is maintained, while in differentiated somatic tissue telomerase expression is repressed. Most tumor cells have a high level of telomerase activity, indicating some loss of regulation in telomerase expression and/or regulation. Moreover tumor cells are often cells which have already undergone many cell cycles before getting malignant and therefore have already short telomeres [29]. As a consequence normal progenitor cells and stem cells, having relatively long telomeres and lower mitotic activity, are not affected to a comparable extent by telomerase inhibition than tumor cells. The most important parameters for susceptibility to telomerase inhibition treatment, namely inherent telomere length, mitotic activity and telomerase expression and activity, are summarized in Figure 4.

cell type	parameters of impact			expected effect
	telomere length	mitotic activity	telomerase activity	
somatic cell	regular shortening	normal	no	no
stem cell	long	low	low	minor
cancer cell	short	high	high	major senescence/apoptosis

Figure 4: Differences in primary telomeres length, mitotic activity and telomerase activity between somatic cells, stem cells and cancer cells are responsible for the high selectivity of telomerase inhibition therapies.

Telomerase inhibition as anticancer therapeutic strategy suffers from one limitation: the lag phase until the telomeres have shortened to the critical limit and cause the onset of cell senescence may take several weeks. This drawback could be limited by clever therapy design or use of additional effects in a combination therapy. Such therapy approaches are reviewed by Shay and Wright [30]. Some experiments with telomerase inhibition noticed a rapid onset of apoptosis [23, 31]. Recent reports propose additional functions of the telomerase enzyme in DNA repair and chromatin maintenance [32]. The addition of cancer cells on telomerase further strengthen the therapeutic concept of telomerase inhibition, because apoptosis occur much earlier than telomere shortening proceed to the critical limit. Parkinson et al published a recent review on the proposed non-canonical functions of telomerase [33].

Inhibition of telomerase is an up-and-coming way of cancer treatment due to the high specificity. In addition there are several further potential targets acting in either stabilization of the telomere end protective structures or proteins (or RNAs) involved in the regulation of telomerase activity.

2. Drugs for telomerase inhibition and telomere related cancer therapy

Noticing the unique role of telomerase in particular in cancer cells induced a lot of research directed to the goal of telomerase inhibition as smart approach for cancer therapy. Numerous studies using very different active ingredients were performed to inhibit telomerase activity. Due to the fact that telomeres needs often many cell cycles to erode to the critical limit a one-time or short-time treatment can only be judged by the impact on telomerase activity. To monitor also the effect on population doubling time or telomere shortening a repeated transient transfection or a stable transfection is essential. The here provided overview aims to mention in the main actives with demonstrated effect on critical endpoints. For a clear

arrangement the listing is structured by classification of the target. An overview for the possible cancer therapeutical concepts following this classification for the target is given in Figure 5. The main points of effect are the catalytic subunit hTERT, the template RNA hTR or the telomere structure, respectively.

Figure 5: Telomerase dependent cancer therapeutical concepts.

Non-competitive hTERT inhibitors and G-quadruplex ligands are small synthetic molecules against a single target. Nucleotide based drugs can be directed with a high specificity against various telomerase affecting targets. In contrast, the concepts of immunotherapy and the use of specific promoters are not acting directly on telomerase activity, but make use of the high expression of telomerase in cancer cells.

2.1 Inhibitors targeting hTERT

The most common approach in inhibiting the telomerase catalytic subunit hTERT is the use of nucleic acid based biologicals. These include siRNA and siRNA expression vectors, ribozymes, which are RNA molecules with specific endonuclease activity, and antisense oligonucleotides. While double stranded siRNA or expression cassettes are more stable, short single stranded RNA or DNA sequences used as antisense oligonucleotides are mainly chemically modified to enhance their stability. This second generation of antisense inhibitors includes phosphorothioate modifications and 2-O'-methyl-RNA capping for enhanced stability against nucleases and improved binding affinity to the target sequence.

Several research groups used siRNA sequences against hTERT and found them to be effective in reduction of telomerase activity *in vitro* [34-39]. The effective doses ranged from 50 to 100nM and resulted in a 50-75% inhibition in dependency on the siRNA sequence and the cell line. Only few reports even describe a reduced cell growth [39, 35, 37].

Shen *et al.* could further provide a proof of concept in an *in vivo* experiment. Colon cancer cells pre-transfected with a functional or mismatch siRNA expression vector were injected into mice. After 6 weeks the treated group had tumors of smaller volume than the control group [34].

A specific anti-hTERT ribozyme was able to reduce mRNA levels and telomerase activity after 48h in 4 ovarian cancer cell lines. The apoptotic rate increased over 7 days up to values of 70-95% [31]. *In vivo* testing of a trans-splicing ribozyme in a peritoneal carcinomatosis

nude mice model led not only to decreased hTERT mRNA levels, 25% of untreated control, but also to a decreased expression of genes involved in antiapoptosis, angiogenesis and metastasis [40].

The antisense oligonucleotide 2'-O'-methyl-RNA (2OMR) against hTERT reduced enzyme activity rate to values <10% of control group in concentrations between 1 and 1.5 μ M in a prostate cancer cell line. Moreover, an 18h exposure to 0.5 μ M 2OMR led to a reduced cell proliferation and enhanced the ratio of apoptotic cells after 48h [23].

A DNA phosphorothioate antisense oligonucleotide (ASODN) targeting hTERT showed promising results in animal experiments using a cervical cancer nude mice model. Daily intratumoral injections of 10nM oligonucleotides resulted in a tumor growth reduction of >50% after 1 week [41].

Beside those nucleotide based biologicals, the small synthetic inhibitor BIBR1532, 2-((E)-3-naphthalene-2-yl-but-2-enoylamino)-benzoic acid, is a potent hTERT inhibitor. BIBR1532 acts as a non-competitive inhibitor of telomerase, which specifically blocks the elongation of DNA [42]. Treatment of different human cancer cell lines *in vitro* resulted in reduced telomere length, inhibition of cell proliferation and cellular senescence. If BIBR1532 pretreated cells were implanted into nude mice, tumor growth was delayed compared to control group. A further treatment of the animal with BIBR1532 did not provide any additional benefit [43].

2.2 hTR inhibitors

Since the telomerase enzyme needs the RNA subunit to be functional, inhibition of the enzymatic activity can also be achieved by targeting hTR. Nucleotide inhibitors comprise short interfering or short hairpin RNA, antisense oligonucleotides and ribozymes, which cause primarily degradation of the template or interfere at least with the assembly of the functional enzyme complex.

The most susceptible region within hTR seems to be the telomere repeat template sequence, which is used as point of attack most commonly [44-47].

In vitro transfections with siRNA using Oligofectamine™ in HCT-15 cells in a concentration range from 71 to 142nM decreased telomerase activity by 75%. Repeated transfection of HeLa cells resulted in a residual enzyme activity of only 5%. The inhibitory effect of this siRNA sequence has also been proven in a human epidermoid and lung cancer cell line. A stable transfection of HeLa cells with a plasmid expressing a hairpin structure against hTR resulted in 5 surviving clones, 3 of them show a decreased telomerase activity between 13 and 57% [44].

Various 2'-O-methyl-RNA or -RNA/DNA chimeric oligomers complementary to the hTR template region were transfected into a human prostate cancer cell line using Lipofectamine™. Two candidate sequences could effectively inhibit telomerase activity with IC₅₀ values of 7-9nM demonstrating the positive effect of phosphorothioate stabilization [45]. In another *in vitro* study 5'-phosphorylated 2'-5' linked oligoadenylate-linked oligonucleotides targeting hTR between residues 76 and 94 were tested in 2 cervical cancer cell lines. Enzyme activity was reduced and the cell viability has been decreased to values of 39% after transfection with 0.5 μ M oligonucleotide and Lipofectamine™. Those effects occurred before the telomere length was affected due to the treatment [48].

The inhibitory potential of several ribozymes directed against sequences in the open loops of the hTR secondary structure was investigated by stable transfection of MCF-7 breast cancer cells with ribozyme expressing vectors. After exposure to 0.5 μ g pDNA combined with the lipidic carrier DOTAP™, only 1 ribozyme, targeted against the template region, efficiently inhibited telomerase. The transfected cells underwent cellular senescence and apoptosis [46].

Another approach in hTR inhibition is the delivery of a mutant-template telomerase RNA (MT-hTR) by a lentiviral system. A markedly decrease in telomerase activity was achieved in

different cancer cell lines resulting in a significantly reduced proliferation rates. Coexpression of a short hairpin RNA against the wild-type hTR template region enhanced the effectivity of the MT-hTR in colon cancer cells *in vitro*. These results were confirmed by an *in vivo* experiment using a nude mice cancer model. The treated animals bore much smaller tumors and showed minimal tumor angiogenesis [49].

Fu et al. compared the inhibitory efficiency of ASODNs targeting either the hTR template region or the hTERT gene and they investigated a putative synergistic effect of a combined treatment. A colon cancer cell line was transfected with 0.2 μ M of various ASODNs and OligofectamineTM and telomerase activity was analyzed after 72h. The anti-hTR-ODN and anti-hTERT-ODN showed similar results regarding the reduction of telomerase activity, about 40% and 45%, and the apoptotic rate, 13,2% and 13,72%, respectively. The combination of both ASODNs reduced the enzyme activity to 19,55% and the apoptotic rate increased to a value of 28,61% [50].

In contrast to the findings in this work, the data published by *Folini et al* show a different result. They also compared an antisense oligonucleotides against hTERT pre-mRNA with a ASODN targeting the template region of hTR. Both oligos were tested in an *in vitro* transfection experiment using the commercial available transfection system DOTAPTM. While 1–1,5 μ M the anti-hTERT 2OMR reduced telomerase activity of values of 10% and lower, 5 μ M of anti-hTR 2OMR was needed to achieve the same effect. Treatment with 0,5 μ M anti-hTERT OMR led to reduced cell growth and an apoptotic rate of about 30%. The anti-hTR OMR did not influence proliferation rate and failed to induce apoptosis in cancer cells [23].

2.3 G-quadruplex ligands

The 3'-G-rich DNA overhangs at the end of human chromosomes, the so-called G-tails, are considered to be essential for the elongation of telomeres. Telomerase need this single stranded, unfolded overhang to add new telomeric repeats to the 3' ends of telomeres. In the presence of cations, the G-tails are able to form certain unusual DNA-structures, among them a G-quadruplex which consists of G-quartets. G-quadruplex ligands are small synthetic molecules that inhibit telomerase activity by stabilizing these quadruplex structures [51, 52]. There is an evidence that these G-quadruplex ligands not only act as inhibitors of hTERT but can also cause uncapping of telomere ends, which is considered to cause a more rapid start of apoptosis than just hTERT inhibition.

Telomestatin was the first potent telomerase inhibitor derived from a natural source. It is a macrocyclic compound, isolated from *Streptomyces anulatus* [53]. Another G-quadruplex ligand is the trisubstituted acridine compound BRACO19, which has shown an anticancer effect *in vitro* and *in vivo* in animal experiments [54]. New promising ligands are isaindigotone derivatives, derived from the root of *Isatis indigotica*, which are thought to have an increased selectivity for the G-quartets and reduced side effects [55]. They remove telomere-associated proteins like POT1, which protect the single stranded G-tails from erosion [56] and the following degradation of the 3'-overhangs leads to an activation of the DNA damage response [57], resulting in the degradation of hTERT and apoptosis [54].

2.4 Telomere-associated proteins

There are several proteins associated with telomeres that are crucial for the protection of telomere ends and thereby influence telomerase function. Telomere repeat binding factors TRF1 and TRF2 are stabilizing double stranded DNA, thus preventing telomere fusion. POT1 binds and protects single stranded DNA, which is essential for telomere elongation.

A modification of the expression of these telomere binding proteins is a strategy in cancer therapy that cannot only influence telomerase function but can also induce uncapping and destabilizing of the telomeric loop structure.

A POT1-TRF1-fusion-protein was able to bind both forms of DNA and could induce mild telomere shortening, if proteins were existent in cis conformation [58]. Upregulation of TRF2 is considered to be involved in multi drug resistance of cancer cells. Chemotherapeutics like adriamycin or vinorelbine induce double strand breaks, TRF2 can bind to this breaking points, reduce the DNA damage response and resulting apoptosis in cancer cells. Inhibition of TRF2 could enhance the efficiency of several anticancer drugs [59]. The poly(ADP-ribose)polymerases (PARPs), also known as tankyrases, are providing a further target for indirect telomerase inhibition. Overexpression of tankyrase leads to enhanced ribosylation of TRF1 and the ribosylated TRF1 can no longer bind and stabilize the duplex telomeric DNA repeats, so there are more single stranded telomeric structures accessible for telomerase. Therefore, downregulation of tankyrase activity could diminish telomere elongation [60].

2.5 Other telomerase inhibitors

Beside the G-quadruplex ligands, small synthetic and nucleic acids based telomerase inhibitors, there is a multiplicity of compounds that can cause a decrease in telomerase activity. Differentiating agents like all-*trans* retinoic acids and DMSO, adriamycin and doxorubicin reduced hTERT mRNA expression. Cancer cells exposed to cisplatin showed a decrease in telomere length while hTERT and hTR levels were not affected. Another family of telomerase inhibitors is the isothiazolone derivatives which could reduce the enzyme activity by interfering with a cysteine in its active center. Other putative inhibitors derived from natural sources are curcumin and a herbal complex based on plant extracts from *Hoelen*, *Angelicae*, *Scutellarriae*, *Glycyrrhizae radix* and other herbs. These other telomerase inhibitors have been reviewed by Beltz and Manfredi [61, 62].

2.6 Further telomere or telomerase related anti-cancer therapies

To complete the overview on telomere and telomerase based cancer therapies the following sections shortly summarize alternative approaches built not upon telomerase inhibition.

2.6.1 Immunotherapy

As the cellular immune response has a higher effect on cancer cells than the humoral immune response has, the application of primary antibodies targeted against certain tumor-associated antigens (TAA) is ineffective in the treatment of cancer. Generating T-lymphocytes that specifically attack tumor cells is a promising approach in the immunotherapy of malignancies. Beside other TAA, hTERT is known to be a MHC class 1-restricted antigen recognized by cytotoxic CD8⁺-T-lymphocytes [63].

Small telomerase-peptides, derived from hTERT, like peptide I540 [64] and H2b telomerase-peptide [65] were used as specific antigens. Activated cytotoxic T-cells were used in the so-called adoptive cell transfer (ACT) therapy based on immunization of nude mice with the antigen, *ex-vivo* selection and expansion of reactive lymphocytes and reinfusion in a tumor-bearing mouse. Tumor growth could be decreased with this method but it still needs to be optimized to avoid a loss of cell selectivity during the expansion step [65, 66].

2.6.2 Conditional gene expression regulated by the telomerase promoter

The selective activation of the hTERT expression in cancer tissue can be used for transcriptional targeting. There are two telomerase-promoter driven cancer therapies currently: viral oncolysis and gene therapies (e.g. expression of suicide genes, tumor suppressor genes, cytokines etc.). Both approaches are frequently studied and reviewed by Fujiwara *et al.* and Gu and Fang, respectively [67, 68]. For example the telomerase-specific replication-competent adenovirus (TRAD) replicated only in cells expressing telomerase and thereby killed predominantly malignant cells. This concept has been proven to be effective *in vivo* while showing no infectious potential [69]. A TNF α expression plasmid regulated either

by hTR or hTERT promoter led to a delay in tumor progression in a xenograft mouse model and an increase in long-term survival [70].

3 Biopharmaceutical problems

Similar like other small synthetic molecules, the telomerase inhibitors telomestatin, isaindigotone derivatives and BIBR1532 suffer from a poor water solubility. So they must be combined with a solubilization enhancer or a drug carrier system to make them bioavailable. Although BRACO19 has a better aqueous solubility, the use of this active is still limited by its very poor permeability. *In vitro* studies demonstrate that BRACO19 is unable to overcome relevant biological barriers in airways and intestinal epithelial cell culture systems even under such relatively favourable conditions *in vitro* [71]. These results were corroborated by an *in vivo* experiment in which oral administration of the active had no therapeutic effect while intraperitoneal injection results in a decrease in tumor volume [54]. In addition, BRACO19 rapidly decomposes in physiological media, depending on pH value and temperature [72]. Figure 6 shows the chemical structures of BIBR1532, isaindigotone and BRACO19 and the main decomposition products of BRACO19.

Figure 6: Chemical structures of small molecule telomerase inhibitors. In red decomposition products of BRACO19 in physiological media according to Taetz *et al.* are indicated [72].

Any options to improve the delivery/bioavailability by some advanced formulation technologies (e.g. solubilizers, penetration enhancers, enzyme inhibitors etc.) appear to be still rather unexplored. Instead, most of the pharmaceutical carrier systems described in the following have originally been designed for some gene therapy purposes.

Because of their predominantly negative charge and large size, the nucleic acid based therapeutics cannot cross cellular membranes by simple passive diffusion. They need a suitable carrier system, such as e.g. virosomes, cationic liposomes and nanoparticles, to reach the cytoplasm or even the nucleus. Another severe problem is the enzymatic degradation of these molecules mediated by endo- and exonucleases. An enhanced nuclease resistance of

antisense nucleotides can be achieved by chemical modifications like phosphorothioate or 2'-O-alkyl modification or the development of peptide nucleic acids [73] and by the use of a gene carrier.

4 Carrier systems

Drug or gene delivery systems, derived from natural or synthetic sources, enable actives to overcome biological barriers, thus enhancing their therapeutic efficiency. In addition, they provide the possibility to control the drug disposition inside the body. Some carriers even allow to change the way of drug administration from a systemic to a local therapy. The carrier system used so far to deliver telomerase inhibitors are summarized in Table 1.

4.1 Biological systems

In gene delivery it is relatively common to follow biomimetic approaches. There are several examples for using attenuated or modified viruses which even have reached clinical trials. A more recent approach is the use of avirulent bacteria as delivery vehicles.

4.1.1 Viruses

Frequently used groups of biological gene vehicles are viruses, among them avirulent strains of adenovirus are the most prominent transfection tools. Viral shuttle plasmids expressing hammerhead ribozymes targeted against the hTERT mRNA could inhibit telomerase activity *in vitro* [31]. *In vivo* transfection by an adeno-associated virus (AAV) which carries the gene coding for tumor necrosis factor related apoptosis-inducing ligand (TRAIL) under control of hTERT promoter led to a decrease in tumor volume in a mouse tumor model. [74].

Another therapeutic approach was the creation of adenoviruses that replicated selective inside cancer cells and thereby killing their host cells. In these oncolytic viruses, the expression of certain viral proteins was put under the control of the hTERT promoter [75].

An efficient lentiviral system was used by Li and coworkers to evaluate the effect of two different approaches of telomerase inhibition: overexpression of mutant hTR and the expression of hairpin siRNA targeting the wild-type hTR template region [49].

The use of viral transfection systems *in vivo* is limited by their side effects. After intratumoral injection, the viruses may distribute and cause adverse reactions in normal tissue [74]. Wang *et al* managed to reduce tissue distribution by increasing the viscosity of the formulation by adding poloxamer 407 [76].

Several viral approaches succeeded to reach pilot studies of gene therapy. However, there are still serious concerns due to few lethal outcomes in clinical trials [77, 78].

4.1.2 Bacteria

Recently, also more complex biological carriers, such as bacteria, have been used as biological gene delivery systems. Bacteria accumulate in tumor tissue using nutritional tropism [79]. Fu, Chu *et al* described avirulent strains of *Salmonella enterica*, equipped with plasmids containing the coding sequences for proteins that induce or enhance apoptosis after cellular uptake. Tumor specificity could be reached by putting these genes under control of the hTERT promoter, resulting in a reduced tumor growth in a mouse cancer model [50].

Another possibility to reach this tumor specific effect is the use of promoters of *S. typhimurium* to control gene transcription. These bacterial promoters are induced by hypoxia, a status that is common in solid tumors and so the expression of the therapeutic effective proteins or peptides is much higher in malignant than in healthy tissue [80].

In spite of their high transfection efficacy and tumor tropism, both viruses and bacteria as biological carriers are burdened with doubts on safety in particular for persons with a weak

immune status like cancer patients undergoing chemotherapy. This situation still stimulates the search for artificial chemistry based delivery systems.

4.2 Chemical Systems

In contrast to the biological gene delivery systems, the chemical carriers are expected to be less immunogenic. Ideally, they can be produced by simple preparations and versatile surface modifications are possible. Another advantage is their higher loading capacity.

Carrier system	Modification	Drug candidate/Target	Endpoint	Reference
4.1.1 Viruses				
Adenovirus	None	pDNA, plasmid expressing a hammerhead ribozyme against hTERT mRNA/ hTERT	<i>In vitro</i> 4 ovarian cancer cell lines Reduced telomerase activity, increased apoptotic rate	[31]
Lentivirus	None	pDNA, plasmid expressing a wild-type hTR and a short hairpin RNA against hTR / hTR	<i>In vivo</i> Murine tumor model Reduced tumor growth, less angiogenesis	[49]
4.1.2 Bacteria				
S. typhimurium	None	pDNA, plasmid expressing proteins that induce apoptosis under the control of the hTERT promoter / hTERT	<i>In vivo</i> Murine tumor model Reduced tumor growth	[50]
4.2.1 Lipids				
Palmitoyl group, GRN163L	None	Antisense oligonucleotide / hTR	Clinical trials Phase I/II	[81]
4.2.1 Liposomes				
DOPE/Cholesterol	Cholesterol modified with 3β[N-(N',N'-dimethylaminoethane) carbamoyl]-group	Antisense oligonucleotide / hTR	<i>In vivo</i> Murine tumor model Reduced tumor growth	[41]
DOPE/Cholesterol	Cholesterol modified with PEI 800	Antisense oligonucleotide / hTERT	<i>In vitro</i> Cervical cancer cell line Reduced cell viability	[82]
4.2.2 Polymers				
Chitosan-coated PLGA-Nanoparticles	positive surface charge	Antisense oligonucleotide / hTR	<i>In vitro</i> Lung cancer cell lines Reduced telomerase activity, telomere shortening, increased apoptotic rate	[83, 84]
Single-walled carbon nanotubes	Modified with N-(6-aminohexyl)carbamate	siRNA / hTERT	<i>In vivo</i> Murine tumor model Reduced tumor growth	[36]

Table 1: Overview about different carriers systems, their surface modifications, delivered actives and the last results published.

4.2.1 Lipid-based carrier systems/liposomes

There are several commercially available, lipidic transfection reagents, optimized for various cell types or nucleic acid based drugs (plasmid DNA, ASODN and siRNA). Although those lipids are optimized gene vehicles for *in vitro* transfection studies, they are not approved for *in vivo* gene therapy because of their unclear toxicity, insufficient stability in physiological conditions and limited transfection efficiency. So there is a strong need for gene delivery systems suitable for clinical applications.

Several telomerase inhibitors were tested in cell culture models by using commercial transfection reagents like Lipofectamine™ [50, 48], siPORT™ [38, 39] or Oligofectamine™ [34, 44]. Although all tested inhibitors showed a sufficient inhibitory potential under optimized *in vitro* conditions, it is not sure that they reach the same efficiency if they are combined with a liposomal carrier suitable for *in vivo* transfection. Further studies with biocompatible vehicles are necessary, before one of those inhibitors could reach clinical trials. Only a few studies are published in which the telomerase inhibitor is combined with such a delivery system. *Chen et al* used liposomes consisting of dioleoylphosphatidylethanolamine (DOPE) and PEI 800-modified cholesterol to deliver antisense oligonucleotides against hTERT. They could reduce cancer cell viability to values below 20% in an *in vitro* experiment. The inhibitory effect was 81fold higher than using naked ASODNs [82]. An *in vivo* study with a similar carrier using 3β-[N-(N',N'-dimethylaminoethane)-carbonyl] cholesterol and DOPE was performed by *Han et al*. The inhibitory rate on the tumor growth in a nude mice model was monitored after intratumoral administration of 10nM anti-hTERT ODN with or without liposomes. Injection of the naked ASODN resulted in an inhibitory rate of 9.2% while the ASODNs with liposomes reached an inhibitory rate of 55.1% [41].

Further cationic liposomal carriers have been developed for different gene therapeutic approaches. As they were all designed to deliver large anionic nucleic acid based drugs over biological barriers, most of them could also provide a suitable carrier system for telomerase inhibitors.

Composition and structure of these liposomes depend on the lipids used and on the nature of the nucleic acid based active. Plasmid DNA was delivered successfully *in vivo* with liposomes consisting of myristyl myristoyl carnitine ester (MMCE) and DOPE. Cellular uptake of pDNA and its stability in physiological fluids was enhanced compared to naked pDNA [85]. Self-assembled lipid nanoparticles have been used to deliver siRNA or ASODNs. *Li et al* complexed calf thymus DNA and protamine with siRNA and combined those complexes with 1-oleoyl-2-[6-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]hexanoyl]-3-trimethylammonium propane (DOTAP™) and cholesterol. Coating the liposomes with distearoylphosphatidylethanolamine (DSPE)-PEG –Anisamide led to an enhanced cellular uptake in cancer cells *in vivo* after intratumoral injection [86]. ASODNs were loaded into DOPE/cholesteryl hemisuccinate (CHEMS)/PEG-DSPE liposomes after mixing with protamine. Treatment with this formulation resulted in an efficient gene downregulation *in vitro* in leucemic cells [87].

4.2.2 Polymer-based carrier systems

For the delivery of nucleic acid based telomerase inhibitors, only two different polymeric carriers have been described so far.

Nanoparticles consisting of a PLGA core, tailorable in size and a positively charged chitosan coating can form stable complexes with DNA or RNA. In contrast to pure PLGA particles, the actives are localized on the surface of the carrier and so the release is much faster [88]. The transfection efficiency of the carrier system is strongly influenced by chitosan content. A

2OMR against hTR was bound to the surface of those particles and the resulting nanoplexes were successfully transfected into different lung cancer cell lines [83].

Delivery of anti-hTERT siRNA with N-(6-aminoethyl)-carbamate modified single-walled carbon nanotubes resulted in a reduced tumor growth *in vivo*. But this carrier system is probably limited by the unclear toxicology and missing biodegradability of carbon nanotubes [36].

Besides these two examples, there are several other gene delivery technologies around, which appear also suitable for telomerase inhibitors. Their exploration for this purpose is however, still pending.

Recently, several PEI derivatives have been described and tested *in vitro* and *in vivo*. PEGylated PEI was reported to form stable polyplexes with pDNA with a lower *in vivo* toxicity [89]. Modifications of branched PEI with acetamid or succinic acid led to a high siRNA transfection efficiency while the toxicity was lower compared to unmodified polymer [90]. PEI derivatives cross-linked with different acrylates mediated high reporter gene expression in the lung or the spleen in mice after retroorbitally injection. These polymers showed only little toxicity in cell culture experiments [91].

PLGA is a biocompatible polymer which degrades into its non-toxic monomers inside the body. Because of its fast endo-lysosomal escape, PLGA is suitable for intracellular delivery of macromolecules like pDNA [92]. Encapsulation of nucleic acid based actives into PLGA micro- or nanocapsules resulted in a sustained release of the drugs. The cumulative release was either depending on the nature of the drug, whereas siRNA and ribozymes had a slower release kinetic than oligonucleotides, and on the lactide : glycolide ratio in the polymer [93].

Another biocompatible polymer used in gene delivery is the natural polysaccharide chitosan. It consists of randomly distributed N-acetylglucosamine and D-glucosamine monomers. More than 65% of these monomers must carry a positive charge to form stable complex with DNA or RNA, so this carrier system is depending on the pH value. Compared to PEI, the transfection efficiency of chitosan started slower but increased over the time and the *in vivo* transfection rate was 3 times lower, but the cytotoxicity *in vitro* was significantly lower [94]. Delivery of siRNA from chitosan nanoparticles mediated reporter gene knockdown with the same efficiency as most commercially available transfection reagents in cultured cells and a successful gene knockdown in the lung after nasal application in mice [95].

More recently developed biodegradable polymers like poly(2-dimethylaminoethylamino) phosphazene (p(DMAEA)-ppz) showed promising results in *in vivo* gene delivery. Polyplexes from pDNA and p(DMAEA)ppz accumulated inside the tumor tissue after i.v. administration in mice, while only a small percentage of the formulation could be found in the lung and the liver [96].

4.3 Targeting

The so-called Enhanced Permeability and Retention (EPR) refers to the observed accumulation of some nanocarriers and macromolecules inside solid tumors, and this effect can be strengthened and specified by application of a targeted delivery system [97]. In order to reach a more distinct uptake into the tumor tissue, some peptide sequences or small molecules that specifically interact with specific or over-expressed surface receptors on malignant cells are coupled to the surfaces of nanomaterials.

Hyaluronic acid-modified liposomes containing an anti-telomerase siRNA have been demonstrated to efficiently target CD44 expressing A549 cells. In addition, complex stability and protection of siRNA was improved by this surface modification [98].

Apart from hyaluronic acid, there are a few other targeting molecules, such as folic acid, transferrin or specific antibodies (e.g. HER-antibody). Polyamidoamine dendrimers modified

with folic acid reached tumor tissue in a higher amount than non-targeted control formulations after intravenous administration [99]. Actives that were delivered by targeted liposomes, equipped with such a specific ligand, accumulated inside the tumor tissue while the level of active inside the healthy tissue was reduced [100]. Coupling of an anti-transferrin receptor single-chain antibody fragment to liposomes enhanced siRNA delivery to primary and metastatic tumors [101]. Potential peptide ligands for specific targeting can be found by the phage display method [102]. Targeted delivery is a method to reach increased amount of drug inside the tumor tissue and a reduction of toxic side effects in normal tissue [99] [100]. However, none of those targeting strategies has yet been applied in a telomerase inhibition approach.

4.4 Local gene therapy by pulmonary application

Another method to increase the amount of the active that reaches the tumor tissue is the loco-regional delivery, such as e.g. the pulmonary application of gene delivery systems as inhalation aerosols.

So-called “stealth” adenoviruses or polyplexes made from siRNA and PEI-PEG block copolymer have proven their transfection efficiency after intratracheal instillation *in vivo* in animals [103, 104], but clinical studies are still pending. Other promising pulmonary gene delivery systems based on either branched biodegradable polyesters or PEG coated super paramagnetic iron oxide nanoparticles (SPIONS), which maintained their transfection ability *in vivo* after nebulization [105, 106].

For convenient pulmonary aerosol delivery, dry powder inhalers are commonly used. Freeze drying of siRNA complexed with chitosan-nanoparticles resulted in stable formulations with good transfection efficiency *in vitro* [107]. Liposomal dispersions containing protamin sulfate and pDNA were transferred into inhalable powders by freeze- or spray-drying, while the spray dried formulation showed better transfection efficiency and better particle properties [108].

Although these methods have not been used yet with formulations containing a telomerase inhibitor, this concept certainly appears transferable.

5 Formulations of telomerase inhibitors

In the following chapter, we want to highlight two formulations developed for the delivery of telomerase inhibitors. Although the drugs used in both cases are antisense oligonucleotide against hTR, the delivery strategies are completely different. While GRN163L, consisting of lipid-modified oligonucleotides is applied via intravenous route, 2OMR complexed with cationic Ch-PLGA nanoparticles can be aerosolized for local pulmonary application.

5.1 GRN163L

A formulation successfully combining a specific telomerase inhibitor and an uptake enhancing delivery system is GRN163L (Geron Corporation, 230 Constitution Drive, Menlo Park, CA 94025 USA), which is currently tested in clinical phase 1 trials against different solid malignancies like breast cancer and non-small cell lung cancer (NSCLC) [109].

GRN163 is a N3'→P5' thiophosphoramidate oligonucleotides, complementary to the template region of the RNA component of human telomerase (hTR). It prevents binding of telomerase to the chromosomal telomere substrate leading to a higher selectivity for cancer cells than a pure hTR inhibitor [110]. Addition of a lipophilic palmitoyl group to the 5' end of the oligonucleotide resulted in a clearly increased transfection efficiency *in vitro* [81, 111]. The antitumoral effect of GRN163L was proven in several *in vivo* studies using xenograft mouse models for different malignancies like non-small cell lung cancer [47] and breast cancer including metastasis [110].

A recently reported finding is that breast cancer cells not only show the “slow response”, i.e. telomere shortening and apoptosis, after treatment, with GRN163L, but also change their morphology while cell growth is reduced [112]. Neither the biochemical mechanism behind this phenomenon nor its meaning for the clinical application of this formulation is completely understood yet.

GRN163L has proven to be a potent telomerase inhibiting formulation showing promising results in clinical trials and has tolerable side effects. It is currently applied via intravenous route, so its selectivity is only due to the oligonucleotide. The amount of the active inside the tumor tissue can be increased by using a tumor-targeted carrier or by developing a local application system.

5.2 Chitosan-PLGA Nanoparticles with 2OMR

Although there have been a lot of proceedings in cancer therapy in recent years, the prognosis in lung cancer remains poor. This kind of malignancy is still one of the leading causes in cancer-related death [113], while the majority of lung cancer patients suffer and die from non-small cell lung cancer (NSCLC).

The presence of telomerase activity was demonstrated in approximately 85% of all human cancers including NSCLC [17] and this finding offers the possibility to develop a new therapeutic approach for NSCLC based on telomerase inhibition.

In a screening experiment of different telomerase inhibitors, among them BIBR1532, BRACO19 and 2 nucleic acid based actives, BRACO19 and a 2'-O-methyl-RNA (2OMR) against hTR turned out to be the most effective inhibitors [114]. In spite of being small organic molecules, BIBR 1532 and BRACO19 are facing several biopharmaceutical problems, as mentioned before in section 3, and so far, there seems to be no formulation to efficiently deliver these molecules *in vivo*. In contrast, there are several technologies described for the delivery of nucleic acid based biologicals, which can probably be applied to the delivery of 2OMR as well.

Figure 7: Uptake behaviour of FAM-OMR and nanoplexes in 2 different lung cancer cell lines, Calu-3 and A549 and primary alveolar epithelial cells over 24h. Red fluorescence: Cellular membranes stained with Rho-RCA, green fluorescence: FAM-OMR. This figure is derived from a publication of *Taetz et al* [83].

For this purpose, nanoparticles consisting of a PLGA core coated with a chitosan layer (Ch-PLGA-NP) were used, which had been originally developed for the transfer of plasmid DNA [115]. Grace to the chitosan coating these nanoparticles are positively charged and form stable “nanoplexes” with anionic oligonucleotides [88]. Size and surface charge of the particles are turnable depending on the process parameters [116], whereas the chitosan content of the formulation has a strong influence on the transfection efficiency of the resulting nanoplexes. A chitosan content of 0.3% turned out to be the most effective [83]. In contrast to the RNA component which is very sensitive against enzymatic degradation, the chitosan-modified PLGA nanoparticles are quiet stable during storage [83]. One major advantage of this formulation is that the components can be stored separately and are mixed just before application. As the active is not incorporated in the polymeric matrix, the release is not limited by the slow erosion of the particle, but it is mainly influenced by the dissociation behaviour of the nanoplex.

Figure 7 gives an overview about the cellular uptake behaviour of the 2OMR- Chitosan-PLGA nanoplexes. While the human lung cancer cell line, Calu-3, was able to take up the carboxyfluorescein (FAM)-labeled OMR (green fluorescence signal) in the absence of nanoparticles, primary human alveolar epithelial cells (hAEpC) and A549 cells could not internalize non-complexed 2OMR, the signal for FAM-OMR remained adsorbed on the cellular membrane. After 6 hours of incubation, nanoplexes could be found in the cytoplasm

of Calu-3 cells, whereas in case of A549 cells, nanoplexes were still co-localized with the membrane. The nanoplexes were mainly localized in the cytoplasm of A549 and Calu-3 cells after 24 hours. For the hAEPc, the nanoplexes could not overcome the cellular membrane [83]

The evaluation of these Ch-PLGA nanoplexes in several *in vitro* transfection studies showed similar transfection efficiencies, determined by means of telomerase inhibition, and a comparable low cytotoxicity compared to lipidic transfection reagents optimized for such cell culture experiments [84, 117].

A decrease in telomerase activity alone does not suffice to overcome the immortality of cancer cells. A more relevant endpoint to demonstrate proof the efficiency of a telomerase inhibitor would be the telomere length and the cell proliferation rate. A long-term treatment of A549 cells over 15 weeks with this nanoplex formulation led to a reduced telomerase activity, in consequence also to telomere shortening and a decrease the in cell population doubling [84]. The results of these experiments are shown in Figure 8.

Figure 8: Telomere shortening and decreased cellular population doubling after long-term treatment with anti-hTR OMR delivered by Ch-PLGA-NP. These data has been published by *Beisner et al.* [84].

Conclusion

There is no question that telomerase and telomeres recent promising targets for cancer therapy. There is a multitude of papers on the biochemical mechanisms, the mode of action, the specific regulation and the importance on the cellular viability and proliferative capacity, elucidating more and more details of this very unique enzyme. Telomerase inhibition has become an end point that is paid attention to in drug screening and drug modelling. Many papers propose actives acting either direct or indirect on telomerase activity. The current publicity due to the recent Nobel Prize awarding for telomerase research will further strengthen the field. Since several proof of concept studies were successful, it is now also the time to address the development of suitable delivery systems.

With regard to the nucleotide actives there is an enormous amount of research in the general field of gene delivery in general. To solve peculiar problems of telomerase inhibition it will be advisable to start with the probably more simple technique of antisense or siRNA inhibition and local treatment. So far the only product in clinical testing is GRN163L from Geron Inc. However, this is an excellent example how a small effort can change the biopharmaceutical profile of an active. Combination of these advanced actives with advanced carrier systems, using recently attained progresses in nanotechnology, could give an impulse for both: cancer therapy and the field of drug delivery, to the benefit of patients.

Acknowledgement

German Cancer Aid Bonn, (project 107694) is thanked for financial support.

References

- [1] C.W. Greider, E.H. Blackburn, A telomeric sequence in the RNA of *Tetrahymena* telomerase required for telomere repeat synthesis. *Nature* 337(6205) (1989) 331-337.
- [2] C.I. Nugent, V. Lundblad, The telomerase reverse transcriptase: components and regulation. *Genes Dev* 12(8) (1998) 1073-1085.
- [3] C.J. Cairney, W.N. Keith, Telomerase redefined: integrated regulation of hTR and hTERT for telomere maintenance and telomerase activity. *Biochimie* 90(1) (2008) 13-23.
- [4] S. Schoeftner, M.A. Blasco, A 'higher order' of telomere regulation: telomere heterochromatin and telomeric RNAs. *Embo J* 28(16) (2009) 2323-2336.
- [5] E.H. Blackburn, Telomere states and cell fates. *Nature* 408(6808) (2000) 53-56.
- [6] J.L. Stern, T.M. Bryan, Telomerase recruitment to telomeres. *Cytogenet Genome Res* 122(3-4) (2008) 243-254.
- [7] E.H. Blackburn, Structure and function of telomeres. *Nature* 350(6319) (1991) 569-573.
- [8] J.D. Griffith, L. Comeau, S. Rosenfield, R.M. Stansel, A. Bianchi, H. Moss, T. de Lange, Mammalian telomeres end in a large duplex loop. *Cell* 97(4) (1999) 503-514.
- [9] T. de Lange, Shelterin: the protein complex that shapes and safeguards human telomeres. *Genes Dev* 19(18) (2005) 2100-2110.
- [10] H.J. Lipps, D. Rhodes, G-quadruplex structures: in vivo evidence and function. *Trends Cell Biol* 19(8) (2009) 414-422.
- [11] C.P. Chiu, C.B. Harley, Replicative senescence and cell immortality: the role of telomeres and telomerase. *Proc Soc Exp Biol Med* 214(2) (1997) 99-106.
- [12] J.W. Shay, W.E. Wright, Hayflick, his limit, and cellular ageing. *Nat Rev Mol Cell Biol* 1(1) (2000) 72-76.
- [13] N.W. Kim, M.A. Piatyszek, K.R. Prowse, C.B. Harley, M.D. West, P.L. Ho, G.M. Coviello, W.E. Wright, S.L. Weinrich, J.W. Shay, Specific association of human telomerase activity with immortal cells and cancer. *Science* 266(5193) (1994) 2011-2015.

- [14] C.M. Counter, H.W. Hirte, S. Bacchetti, C.B. Harley, Telomerase activity in human ovarian carcinoma. *Proc Natl Acad Sci U S A* 91(8) (1994) 2900-2904.
- [15] E. Hiyama, L. Gollahon, T. Kataoka, K. Kuroi, T. Yokoyama, A.F. Gazdar, K. Hiyama, M.A. Piatyszek, J.W. Shay, Telomerase activity in human breast tumors. *J Natl Cancer Inst* 88(2) (1996) 116-122.
- [16] E. Hiyama, T. Yokoyama, N. Tatsumoto, K. Hiyama, Y. Imamura, Y. Murakami, T. Kodama, M.A. Piatyszek, J.W. Shay, Y. Matsuura, Telomerase activity in gastric cancer. *Cancer Res* 55(15) (1995) 3258-3262.
- [17] K. Hiyama, E. Hiyama, S. Ishioka, M. Yamakido, K. Inai, A.F. Gazdar, M.A. Piatyszek, J.W. Shay, Telomerase activity in small-cell and non-small-cell lung cancers. *J Natl Cancer Inst* 87(12) (1995) 895-902.
- [18] M.M. Kim, M.A. Rivera, I.L. Botchkina, R. Shalaby, A.D. Thor, E.H. Blackburn, A low threshold level of expression of mutant-template telomerase RNA inhibits human tumor cell proliferation. *Proc Natl Acad Sci U S A* 98(14) (2001) 7982-7987.
- [19] E. Gonzalez-Suarez, E. Samper, J.M. Flores, M.A. Blasco, Telomerase-deficient mice with short telomeres are resistant to skin tumorigenesis. *Nat Genet* 26(1) (2000) 114-117.
- [20] A. Goldkorn, E.H. Blackburn, Assembly of mutant-template telomerase RNA into catalytically active telomerase ribonucleoprotein that can act on telomeres is required for apoptosis and cell cycle arrest in human cancer cells. *Cancer Res* 66(11) (2006) 5763-5771.
- [21] P. Gandellini, M. Folini, R. Bandiera, M. De Cesare, M. Binda, S. Veronese, M.G. Daidone, F. Zunino, N. Zaffaroni, Down-regulation of human telomerase reverse transcriptase through specific activation of RNAi pathway quickly results in cancer cell growth impairment. *Biochem Pharmacol* 73(11) (2007) 1703-1714.
- [22] L. Zou, P. Zhang, C. Luo, Z. Tu, shRNA-targeted hTERT suppress cell proliferation of bladder cancer by inhibiting telomerase activity. *Cancer Chemother Pharmacol* 57(3) (2006) 328-334.
- [23] M. Folini, C. Brambilla, R. Villa, P. Gandellini, S. Vignati, F. Paduano, M.G. Daidone, N. Zaffaroni, Antisense oligonucleotide-mediated inhibition of hTERT, but not hTERC, induces rapid cell growth decline and apoptosis in the absence of telomere shortening in human prostate cancer cells. *Eur J Cancer* 41(4) (2005) 624-634.
- [24] M. Nosrati, S. Li, S. Bagheri, D. Ginzinger, E.H. Blackburn, R.J. Debs, M. Kashani-Sabet, Antitumor activity of systemically delivered ribozymes targeting murine telomerase RNA. *Clin Cancer Res* 10(15) (2004) 4983-4990.
- [25] G.M. Clark, C.K. Osborne, D. Levitt, F. Wu, N.W. Kim, Telomerase activity and survival of patients with node-positive breast cancer. *J Natl Cancer Inst* 89(24) (1997) 1874-1881.
- [26] C.W. Greider, Telomerase activity, cell proliferation, and cancer. *Proc Natl Acad Sci U S A* 95(1) (1998) 90-92.
- [27] S. Kyo, M. Takakura, T. Kohama, M. Inoue, Telomerase activity in human endometrium. *Cancer Res* 57(4) (1997) 610-614.
- [28] R.D. Ramirez, W.E. Wright, J.W. Shay, R.S. Taylor, Telomerase activity concentrates in the mitotically active segments of human hair follicles. *J Invest Dermatol* 108(1) (1997) 113-117.
- [29] J.W. Shay, W.E. Wright, Telomerase therapeutics for cancer: challenges and new directions. *Nat Rev Drug Discov* 5(7) (2006) 577-584.
- [30] J.W. Shay, W.E. Wright, Telomerase: a target for cancer therapeutics. *Cancer Cell* 2(4) (2002) 257-265.
- [31] G. Saretzki, A. Ludwig, T. von Zglinicki, I.B. Runnebaum, Ribozyme-mediated telomerase inhibition induces immediate cell loss but not telomere shortening in ovarian cancer cells. *Cancer Gene Ther* 8(10) (2001) 827-834.

- [32] K. Masutomi, R. Possemato, J.M. Wong, J.L. Currier, Z. Tothova, J.B. Manola, S. Ganesan, P.M. Lansdorp, K. Collins, W.C. Hahn, The telomerase reverse transcriptase regulates chromatin state and DNA damage responses. *Proc Natl Acad Sci U S A* 102(23) (2005) 8222-8227.
- [33] E.K. Parkinson, C. Fitchett, B. Cereser, Dissecting the non-canonical functions of telomerase. *Cytogenet Genome Res* 122(3-4) (2008) 273-280.
- [34] Y. Shen, Y.W. Zhang, Z.X. Zhang, Z.H. Miao, J. Ding, hTERT-targeted RNA interference inhibits tumorigenicity and motility of HCT116 cells. *Cancer Biol Ther* 7(2) (2008) 228-236.
- [35] Y. Xia, R.X. Lin, S.J. Zheng, Y. Yang, X.C. Bo, D.Y. Zhu, S.Q. Wang, Effective siRNA targets screening for human telomerase reverse transcriptase. *World J Gastroenterol* 11(16) (2005) 2497-2501.
- [36] Z. Zhang, X. Yang, Y. Zhang, B. Zeng, S. Wang, T. Zhu, R.B. Roden, Y. Chen, R. Yang, Delivery of telomerase reverse transcriptase small interfering RNA in complex with positively charged single-walled carbon nanotubes suppresses tumor growth. *Clin Cancer Res* 12(16) (2006) 4933-4939.
- [37] M.A. Shammash, H. Koley, R.B. Batchu, R.C. Bertheau, A. Protopopov, N.C. Munshi, R.K. Goyal, Telomerase inhibition by siRNA causes senescence and apoptosis in Barrett's adenocarcinoma cells: mechanism and therapeutic potential. *Mol Cancer* 4 (2005) 24.
- [38] J.N. Zheng, Y.F. Sun, D.S. Pei, J.J. Liu, J.C. Chen, W. Li, X.Q. Sun, Q.D. Shi, R.F. Han, T.X. Ma, Inhibition of proliferation and induction of apoptosis in human renal carcinoma cells by anti-telomerase small interfering RNAs. *Acta Biochim Biophys Sin (Shanghai)* 38(7) (2006) 500-506.
- [39] K. Kurvinen, S. Syrjanen, B. Johansson, Long-term suppression of telomerase expression in HeLa cell clones, transfected with an expression vector carrying siRNA targeting hTERT mRNA. *Int J Oncol* 29(1) (2006) 279-288.
- [40] M.S. Song, J.S. Jeong, K.S. Cho, S.W. Lee, Gene expression responses in vivo by human telomerase reverse transcriptase (hTERT)-targeting trans-splicing ribozyme. *Exp Mol Med* 39(6) (2007) 722-732.
- [41] M. Han, J.L. Chen, Y. Hu, C.L. He, W.P. Shuai, J.H. Yu, H.L. Chen, W.Q. Liang, T. Mayumi, N. Shinsaku, J.Q. Gao, In vitro and in vivo tumor suppressive activity induced by human telomerase transcriptase-targeting antisense oligonucleotides mediated by cationic liposomes. *J Biosci Bioeng* 106(3) (2008) 243-247.
- [42] E. Pascolo, C. Wenz, J. Lingner, N. Hael, H. Priepe, I. Kauffmann, P. Garin-Chesa, W.J. Rettig, K. Damm, A. Schnapp, Mechanism of human telomerase inhibition by BIBR1532, a synthetic, non-nucleosidic drug candidate. *J Biol Chem* 277(18) (2002) 15566-15572.
- [43] K. Damm, U. Hemmann, P. Garin-Chesa, N. Hael, I. Kauffmann, H. Priepe, C. Niestroj, C. Daiber, B. Enenkel, B. Guilliard, I. Lauritsch, E. Muller, E. Pascolo, G. Sauter, M. Pantic, U.M. Martens, C. Wenz, J. Lingner, N. Kraut, W.J. Rettig, A. Schnapp, A highly selective telomerase inhibitor limiting human cancer cell proliferation. *Embo J* 20(24) (2001) 6958-6968.
- [44] B.A. Kosciolk, K. Kalantidis, M. Tabler, P.T. Rowley, Inhibition of telomerase activity in human cancer cells by RNA interference. *Mol Cancer Ther* 2(3) (2003) 209-216.
- [45] A.N. Elayadi, A. Demieville, E.V. Wancewicz, B.P. Monia, D.R. Corey, Inhibition of telomerase by 2'-O-(2-methoxyethyl) RNA oligomers: effect of length, phosphorothioate substitution and time inside cells. *Nucleic Acids Res* 29(8) (2001) 1683-1689.
- [46] M. Yeo, S.Y. Rha, H.C. Jeung, S.X. Hu, S.H. Yang, Y.S. Kim, S.W. An, H.C. Chung, Attenuation of telomerase activity by hammerhead ribozyme targeting human telomerase RNA induces growth retardation and apoptosis in human breast tumor cells. *Int J Cancer* 114(3) (2005) 484-489.

- [47] Z.G. Dikmen, G.C. Gellert, S. Jackson, S. Gryaznov, R. Tressler, P. Dogan, W.E. Wright, J.W. Shay, In vivo inhibition of lung cancer by GRN163L: a novel human telomerase inhibitor. *Cancer Res* 65(17) (2005) 7866-7873.
- [48] N. Yatabe, S. Kyo, S. Kondo, T. Kanaya, Z. Wang, Y. Maida, M. Takakura, M. Nakamura, M. Tanaka, M. Inoue, 2-5A antisense therapy directed against human telomerase RNA inhibits telomerase activity and induces apoptosis without telomere impairment in cervical cancer cells. *Cancer Gene Ther* 9(7) (2002) 624-630.
- [49] S. Li, J.E. Rosenberg, A.A. Donjacour, I.L. Botchkina, Y.K. Hom, G.R. Cunha, E.H. Blackburn, Rapid inhibition of cancer cell growth induced by lentiviral delivery and expression of mutant-template telomerase RNA and anti-telomerase short-interfering RNA. *Cancer Res* 64(14) (2004) 4833-4840.
- [50] W. Fu, L. Chu, X. Han, X. Liu, D. Ren, Synergistic antitumoral effects of human telomerase reverse transcriptase-mediated dual-apoptosis-related gene vector delivered by orally attenuated *Salmonella enterica* Serovar Typhimurium in murine tumor models. *J Gene Med* 10(6) (2008) 690-701.
- [51] V.L. Makarov, Y. Hirose, J.P. Langmore, Long G tails at both ends of human chromosomes suggest a C strand degradation mechanism for telomere shortening. *Cell* 88(5) (1997) 657-666.
- [52] A. De Cian, G. Cristofari, P. Reichenbach, E. De Lemos, D. Monchaud, M.P. Teulade-Fichou, K. Shin-Ya, L. Lacroix, J. Lingner, J.L. Mergny, Reevaluation of telomerase inhibition by quadruplex ligands and their mechanisms of action. *Proc Natl Acad Sci U S A* 104(44) (2007) 17347-17352.
- [53] J.H. Tan, L.Q. Gu, J.Y. Wu, Design of selective G-quadruplex ligands as potential anticancer agents. *Mini Rev Med Chem* 8(11) (2008) 1163-1178.
- [54] A.M. Burger, F. Dai, C.M. Schultes, A.P. Reszka, M.J. Moore, J.A. Double, S. Neidle, The G-quadruplex-interactive molecule BRACO-19 inhibits tumor growth, consistent with telomere targeting and interference with telomerase function. *Cancer Res* 65(4) (2005) 1489-1496.
- [55] J.H. Tan, T.M. Ou, J.Q. Hou, Y.J. Lu, S.L. Huang, H.B. Luo, J.Y. Wu, Z.S. Huang, K.Y. Wong, L.Q. Gu, Isaindigotone derivatives: a new class of highly selective ligands for telomeric G-quadruplex DNA. *J Med Chem* 52(9) (2009) 2825-2835.
- [56] D. Gomez, M.F. O'Donohue, T. Wenner, C. Douarre, J. Macadre, P. Koebel, M.J. Giraud-Panis, H. Kaplan, A. Kolkes, K. Shin-ya, J.F. Riou, The G-quadruplex ligand telomestatin inhibits POT1 binding to telomeric sequences in vitro and induces GFP-POT1 dissociation from telomeres in human cells. *Cancer Res* 66(14) (2006) 6908-6912.
- [57] L. Kelland, Targeting the limitless replicative potential of cancer: the telomerase/telomere pathway. *Clin Cancer Res* 13(17) (2007) 4960-4963.
- [58] K.T. Etheridge, S.A. Compton, K.S. Barrientos, S. Ozgur, J.D. Griffith, C.M. Counter, Tethering telomeric double- and single-stranded DNA-binding proteins inhibits telomere elongation. *J Biol Chem* 283(11) (2008) 6935-6941.
- [59] H. Ning, T. Li, L. Zhao, J. Li, J. Liu, Z. Liu, D. Fan, TRF2 promotes multidrug resistance in gastric cancer cells. *Cancer Biol Ther* 5(8) (2006) 950-956.
- [60] A. Smogorzewska, T. de Lange, Regulation of telomerase by telomeric proteins. *Annu Rev Biochem* 73 (2004) 177-208.
- [61] L.A. Beltz, The 'other' telomerase inhibitors: non-G-quadruplex interactive agent, non-antisense, non-reverse transcriptase telomerase inhibitors. *Curr Med Chem Anticancer Agents* 2(5) (2002) 589-603.
- [62] L.A. Beltz, Manfredi, K. P. , "An Update on the Other Telomerase Inhibitors: Non-G-Quadruplex Interactive Agent, Non-Antisense, Non-Reverse Transcriptase Telomerase Inhibitors." *Medicinal Chemistry Reviews*(2) (2005) 325-344, 319p.

- [63] S.A. Rosenberg, Progress in human tumour immunology and immunotherapy. *Nature* 411(6835) (2001) 380-384.
- [64] R.H. Vonderheide, W.C. Hahn, J.L. Schultze, L.M. Nadler, The telomerase catalytic subunit is a widely expressed tumor-associated antigen recognized by cytotoxic T lymphocytes. *Immunity* 10(6) (1999) 673-679.
- [65] J. Schmidt, E. Ryschich, E. Sievers, I.G. Schmidt-Wolf, M.W. Buchler, A. Marten, Telomerase-specific T-cells kill pancreatic tumor cells in vitro and in vivo. *Cancer* 106(4) (2006) 759-764.
- [66] H. Hassanin, S. Serba, J. Schmidt, A. Marten, Ex vivo expanded telomerase-specific T cells are effective in an orthotopic mouse model for pancreatic adenocarcinoma. *Clin Exp Immunol* 158(1) (2009) 125-132.
- [67] T. Fujiwara, Y. Urata, N. Tanaka, Telomerase-specific oncolytic virotherapy for human cancer with the hTERT promoter. *Curr Cancer Drug Targets* 7(2) (2007) 191-201.
- [68] J. Gu, B. Fang, Telomerase promoter-driven cancer gene therapy. *Cancer Biol Ther* 2(4 Suppl 1) (2003) S64-70.
- [69] T. Kawashima, S. Kagawa, N. Kobayashi, Y. Shirakiya, T. Umeoka, F. Teraishi, M. Taki, S. Kyo, N. Tanaka, T. Fujiwara, Telomerase-specific replication-selective virotherapy for human cancer. *Clin Cancer Res* 10(1 Pt 1) (2004) 285-292.
- [70] C. Dufes, W.N. Keith, A. Bilsland, I. Proutski, I.F. Uchegbu, A.G. Schatzlein, Synthetic anticancer gene medicine exploits intrinsic antitumor activity of cationic vector to cure established tumors. *Cancer Res* 65(18) (2005) 8079-8084.
- [71] S. Taetz, C. Baldes, T.E. Murdter, E. Kleideiter, K. Piotrowska, U. Bock, E. Haltner-Ukomadu, J. Mueller, H. Huwer, U.F. Schaefer, U. Klotz, C.M. Lehr, Biopharmaceutical characterization of the telomerase inhibitor BRACO19. *Pharm Res* 23(5) (2006) 1031-1037.
- [72] S. Taetz, T.E. Murdter, J. Zapp, S. Boettcher, C. Baldes, E. Kleideiter, K. Piotrowska, U.F. Schaefer, U. Klotz, C.M. Lehr, Decomposition of the telomere-targeting agent BRACO19 in physiological media results in products with decreased inhibitory potential. *Int J Pharm* 357(1-2) (2008) 6-14.
- [73] A. Kalota, S.E. Shetzline, A.M. Gewirtz, Progress in the development of nucleic acid therapeutics for cancer. *Cancer Biol Ther* 3(1) (2004) 4-12.
- [74] Y. Wang, F. Huang, H. Cai, S. Zhong, X. Liu, W.S. Tan, Potent antitumor effect of TRAIL mediated by a novel adeno-associated viral vector targeting to telomerase activity for human hepatocellular carcinoma. *J Gene Med* 10(5) (2008) 518-526.
- [75] T.G. Huang, M.J. Savontaus, K. Shinozaki, B.V. Sauter, S.L. Woo, Telomerase-dependent oncolytic adenovirus for cancer treatment. *Gene Ther* 10(15) (2003) 1241-1247.
- [76] Y. Wang, S. Liu, C.Y. Li, F. Yuan, A novel method for viral gene delivery in solid tumors. *Cancer Res* 65(17) (2005) 7541-7545.
- [77] E. Marshall, Gene therapy death prompts review of adenovirus vector. *Science* 286(5448) (1999) 2244-2245.
- [78] S.E. Raper, N. Chirmule, F.S. Lee, N.A. Wivel, A. Bagg, G.P. Gao, J.M. Wilson, M.L. Batshaw, Fatal systemic inflammatory response syndrome in a ornithine transcarbamylase deficient patient following adenoviral gene transfer. *Mol Genet Metab* 80(1-2) (2003) 148-158.
- [79] Y. Seow, M.J. Wood, Biological gene delivery vehicles: beyond viral vectors. *Mol Ther* 17(5) (2009) 767-777.
- [80] N. Arrach, M. Zhao, S. Porwollik, R.M. Hoffman, M. McClelland, Salmonella promoters preferentially activated inside tumors. *Cancer Res* 68(12) (2008) 4827-4832.
- [81] B.S. Herbert, G.C. Gellert, A. Hochreiter, K. Pongracz, W.E. Wright, D. Zielinska, A.C. Chin, C.B. Harley, J.W. Shay, S.M. Gryaznov, Lipid modification of GRN163, an N3'-->P5' thio-phosphoramidate oligonucleotide, enhances the potency of telomerase inhibition. *Oncogene* 24(33) (2005) 5262-5268.

- [82] J.L. Chen, Y. Hu, W.P. Shuai, H.L. Chen, W.Q. Liang, J.Q. Gao, Telomerase-targeting antisense oligonucleotides carried by polycation liposomes enhance the growth inhibition effect on tumor cells. *J Biomed Mater Res B Appl Biomater* 89B(2) (2009) 362-368.
- [83] S. Taetz, N. Nafee, J. Beisner, K. Piotrowska, C. Baldes, T.E. Murdter, H. Huwer, M. Schneider, U.F. Schaefer, U. Klotz, C.M. Lehr, The influence of chitosan content in cationic chitosan/PLGA nanoparticles on the delivery efficiency of antisense 2'-O-methyl-RNA directed against telomerase in lung cancer cells. *Eur J Pharm Biopharm* 72(2) (2009) 358-369.
- [84] J. Beisner, M. Dong, S. Taetz, N. Nafee, E.U. Griesse, U. Schaefer, C.M. Lehr, U. Klotz, T.E. Murdter, Nanoparticle mediated delivery of 2'-O-methyl-RNA leads to efficient telomerase inhibition and telomere shortening in human lung cancer cells. *Lung Cancer* (2009).
- [85] D. Deshpande, P. Blezinger, R. Pillai, J. Duguid, B. Freimark, A. Rolland, Target specific optimization of cationic lipid-based systems for pulmonary gene therapy. *Pharm Res* 15(9) (1998) 1340-1347.
- [86] S.D. Li, S. Chono, L. Huang, Efficient gene silencing in metastatic tumor by siRNA formulated in surface-modified nanoparticles. *J Control Release* 126(1) (2008) 77-84.
- [87] Y. Jin, S. Liu, B. Yu, S. Golan, C.G. Koh, J. Yang, L. Huynh, X. Yang, J. Pang, N. Muthusamy, K.K. Chan, J.C. Byrd, Y. Talmon, L.J. Lee, R.J. Lee, G. Marcucci, Targeted Delivery of Antisense Oligodeoxynucleotide by Transferrin Conjugated pH-Sensitive Lipopolyplex Nanoparticles: A Novel Oligonucleotide-Based Therapeutic Strategy in Acute Myeloid Leukemia. *Mol Pharm* (2009).
- [88] M.N. Kumar, S.S. Mohapatra, X. Kong, P.K. Jena, U. Bakowsky, C.M. Lehr, Cationic poly(lactide-co-glycolide) nanoparticles as efficient in vivo gene transfection agents. *J Nanosci Nanotechnol* 4(8) (2004) 990-994.
- [89] T. Merdan, K. Kunath, H. Petersen, U. Bakowsky, K.H. Voigt, J. Kopecek, T. Kissel, PEGylation of poly(ethylene imine) affects stability of complexes with plasmid DNA under in vivo conditions in a dose-dependent manner after intravenous injection into mice. *Bioconjug Chem* 16(4) (2005) 785-792.
- [90] A. Zintchenko, A. Philipp, A. Dehshahri, E. Wagner, Simple modifications of branched PEI lead to highly efficient siRNA carriers with low toxicity. *Bioconjug Chem* 19(7) (2008) 1448-1455.
- [91] M. Thomas, J.J. Lu, C. Zhang, J. Chen, A.M. Klibanov, Identification of novel superior polycationic vectors for gene delivery by high-throughput synthesis and screening of a combinatorial library. *Pharm Res* 24(8) (2007) 1564-1571.
- [92] J. Panyam, W.Z. Zhou, S. Prabha, S.K. Sahoo, V. Labhasetwar, Rapid endo-lysosomal escape of poly(DL-lactide-co-glycolide) nanoparticles: implications for drug and gene delivery. *Faseb J* 16(10) (2002) 1217-1226.
- [93] A. Khan, M. Benboubetra, P.Z. Sayyed, K.W. Ng, S. Fox, G. Beck, I.F. Benter, S. Akhtar, Sustained polymeric delivery of gene silencing antisense ODNs, siRNA, DNazymes and ribozymes: in vitro and in vivo studies. *J Drug Target* 12(6) (2004) 393-404.
- [94] M. Koping-Hoggard, I. Tubulekas, H. Guan, K. Edwards, M. Nilsson, K.M. Varum, P. Artursson, Chitosan as a nonviral gene delivery system. Structure-property relationships and characteristics compared with polyethylenimine in vitro and after lung administration in vivo. *Gene Ther* 8(14) (2001) 1108-1121.
- [95] K.A. Howard, U.L. Rahbek, X. Liu, C.K. Damgaard, S.Z. Glud, M.O. Andersen, M.B. Hovgaard, A. Schmitz, J.R. Nyengaard, F. Besenbacher, J. Kjems, RNA interference in vitro and in vivo using a novel chitosan/siRNA nanoparticle system. *Mol Ther* 14(4) (2006) 476-484.
- [96] H.K. de Wolf, M. de Raad, C. Snel, M.J. van Steenberghe, M.H. Fens, G. Storm, W.E. Hennink, Biodegradable poly(2-dimethylamino ethylamino)phosphazene for in vivo gene

- delivery to tumor cells. Effect of polymer molecular weight. *Pharm Res* 24(8) (2007) 1572-1580.
- [97] R. Duncan, Polymer conjugates for tumour targeting and intracytoplasmic delivery. The EPR effect as a common gateway? *Pharm Sci Technolo Today* 2(11) (1999) 441-449.
- [98] S. Taetz, A. Bochot, C. Surace, S. Arpicco, J.M. Renoir, U.F. Schaefer, V. Marsaud, S. Kerdine-Roemer, C.M. Lehr, E. Fattal, Hyaluronic acid-modified DOTAP/DOPE liposomes for the targeted delivery of anti-telomerase siRNA to CD44-expressing lung cancer cells. *Oligonucleotides* 19(2) (2009) 103-116.
- [99] J.F. Kukowska-Latallo, K.A. Candido, Z. Cao, S.S. Nigavekar, I.J. Majoros, T.P. Thomas, L.P. Balogh, M.K. Khan, J.R. Baker, Jr., Nanoparticle targeting of anticancer drug improves therapeutic response in animal model of human epithelial cancer. *Cancer Res* 65(12) (2005) 5317-5324.
- [100] D.K. Chang, C.T. Lin, C.H. Wu, H.C. Wu, A novel peptide enhances therapeutic efficacy of liposomal anti-cancer drugs in mice models of human lung cancer. *PLoS One* 4(1) (2009) e4171.
- [101] K.F. Pirollo, G. Zon, A. Rait, Q. Zhou, W. Yu, R. Hogrefe, E.H. Chang, Tumor-targeting nanoimmunoliposome complex for short interfering RNA delivery. *Hum Gene Ther* 17(1) (2006) 117-124.
- [102] R.J. Giordano, J.K. Edwards, R.M. Tudor, W. Arap, R. Pasqualini, Combinatorial ligand-directed lung targeting. *Proc Am Thorac Soc* 6(5) (2009) 411-415.
- [103] M.A. Croyle, N. Chirmule, Y. Zhang, J.M. Wilson, "Stealth" adenoviruses blunt cell-mediated and humoral immune responses against the virus and allow for significant gene expression upon readministration in the lung. *J Virol* 75(10) (2001) 4792-4801.
- [104] O.M. Merkel, A. Beyerle, D. Librizzi, A. Pfestroff, T.M. Behr, B. Sproat, P.J. Barth, T. Kissel, Nonviral siRNA delivery to the lung: investigation of PEG-PEI polyplexes and their in vivo performance. *Mol Pharm* 6(4) (2009) 1246-1260.
- [105] J. Nguyen, R. Reul, T. Betz, E. Dayyoub, T. Schmehl, T. Gessler, U. Bakowsky, W. Seeger, T. Kissel, Nanocomposites of lung surfactant and biodegradable cationic nanoparticles improve transfection efficiency to lung cells. *J Control Release* 140(1) (2009) 47-54.
- [106] P. Dames, B. Gleich, A. Flemmer, K. Hajek, N. Seidl, F. Wiekhorst, D. Eberbeck, I. Bittmann, C. Bergemann, T. Weyh, L. Trahms, J. Rosenecker, C. Rudolph, Targeted delivery of magnetic aerosol droplets to the lung. *Nat Nanotechnol* 2(8) (2007) 495-499.
- [107] M.O. Andersen, K.A. Howard, S.R. Paludan, F. Besenbacher, J. Kjems, Delivery of siRNA from lyophilized polymeric surfaces. *Biomaterials* 29(4) (2008) 506-512.
- [108] P.C. Seville, I.W. Kellaway, J.C. Birchall, Preparation of dry powder dispersions for non-viral gene delivery by freeze-drying and spray-drying. *J Gene Med* 4(4) (2002) 428-437.
- [109] A. Molckovsky, L.L. Siu, First-in-class, first-in-human phase I results of targeted agents: Highlights of the 2008 American Society of Clinical Oncology meeting. *J Hematol Oncol* 1 (2008) 20.
- [110] A.E. Hochreiter, H. Xiao, E.M. Goldblatt, S.M. Gryaznov, K.D. Miller, S. Badve, G.W. Sledge, B.S. Herbert, Telomerase template antagonist GRN163L disrupts telomere maintenance, tumor growth, and metastasis of breast cancer. *Clin Cancer Res* 12(10) (2006) 3184-3192.
- [111] M.W. Djojotubroto, A.C. Chin, N. Go, S. Schaetzlein, M.P. Manns, S. Gryaznov, C.B. Harley, K.L. Rudolph, Telomerase antagonists GRN163 and GRN163L inhibit tumor growth and increase chemosensitivity of human hepatoma. *Hepatology* 42(5) (2005) 1127-1136.
- [112] E.M. Goldblatt, E.R. Gentry, M.J. Fox, S.M. Gryaznov, C. Shen, B.S. Herbert, The telomerase template antagonist GRN163L alters MDA-MB-231 breast cancer cell morphology, inhibits growth, and augments the effects of paclitaxel. *Mol Cancer Ther* 8(7) (2009) 2027-2035.

- [113] A. Jemal, R. Siegel, E. Ward, Y. Hao, J. Xu, M.J. Thun, Cancer statistics, 2009. *CA Cancer J Clin* 59(4) (2009) 225-249.
- [114] E. Kleideiter, K. Piotrowska, U. Klotz, Screening of telomerase inhibitors. *Methods Mol Biol* 405 (2007) 167-180.
- [115] M.N.V. Ravi Kumar, U. Bakowsky, C.M. Lehr, Preparation and characterization of cationic PLGA nanospheres as DNA carriers. *Biomaterials* 2004, pp. pp. 1771-1777.
- [116] N. Nafee, S. Taetz, M. Schneider, U.F. Schaefer, C.M. Lehr, Chitosan-coated PLGA nanoparticles for DNA/RNA delivery: effect of the formulation parameters on complexation and transfection of antisense oligonucleotides. *Nanomedicine* 3(3) (2007) 173-183.
- [117] J. Beisner, M. Dong, S. Taetz, K. Piotrowska, E. Kleideiter, G. Friedel, U. Schaefer, C.M. Lehr, U. Klotz, T.E. Murdter, Efficient telomerase inhibition in human non-small cell lung cancer cells by liposomal delivery of 2'-O-methyl-RNA. *J Pharm Sci* 98(5) (2009) 1765-1774.