Nuclisome – targeting the tumor cell nucleus

Lars Gedda[1] and Katarina Edwards[2, 3]

[1]*Department of Radiology, Oncology and Radiation Sciences, Unit of Biomedical Radiation Sciences, Rudbeck Laboratory, Uppsala University, 751 85 Uppsala, Sweden.*

[2]*Department of Physical and Analytical Chemistry, Uppsala University, P.O. Box 579, 751 23, Uppsala, Sweden,*

[3] *FRIAS, School of Soft Matter Research, University of Freiburg, Albertstraße 19, 79104 Freiburg, Germany*

Corresponding author: Lars Gedda, Lars.Gedda@bms.uu.se, +46 18 4713431, FAX +46 18 4713432

Keywords: Auger, intercalator, liposome, radionuclide, DNA

Abstract

The Nuclisome concept builds on a novel two-step targeting strategy with the aim to deliver short-range Auger-electron emitting radionuclides to nuclear DNA of tumor cells. The concept is based on the use of Nuclisome-particles, i.e., tumor-targeted PEG-stabilized liposomes loaded with a unique DNA-intercalating compound that enables specific and effective delivery of radionuclides to DNA. The specific and potent two-step targeting leads to eradication of tumor cells while toxicity to normal organs is reduced to a minimum. Results of *in vitro* and *in vivo* studies point towards the Nuclisome concept as a promising strategy for treatment of small tumor masses and, in particular, elimination of spread single cells and micrometastases.

Introduction

The efficacy of conventional cancer chemotherapy is often limited by severe side effects that preclude high drug levels from being reached at the tumor site. Targeted drug delivery via liposomes attempts to increase the specificity, and thus minimize the dose-limiting side effects, by encapsulating the cytotoxic agent in liposomes and attaching tumor-specific ligands to the liposome surface. However, despite some progress the strategy with targeted liposomes loaded with conventional anticancer drugs has so far only had limited success [1,2]. The Nuclisome concept offers a means to boost the anti-tumor effect while toxicity to normal tissue is kept low. Thereby the risk/benefit profile of the drug is significantly improved as compared to that of standard cytotoxic drugs encapsulated in targeted liposomes.

The Nuclisome concept utilizes liposomal carriers in combination with a two-step targeting principle, and aims at the delivery of Auger-electron emitting radionuclides to DNA in the nucleus of tumor cells. The rationale behind the use of Auger-electron emitting radionuclides is to exploit the biological Auger effect caused by local energy absorption of multiple low-energy electrons to create complex double-strand breaks (DSB) in the DNA [3]. Due to the fact that most of the energy released from Auger electrons is deposited within a small volume of a few cubic nanometers, it is insufficient to target just the tumor cells—the DNA itself needs to be targeted. In fact, to obtain full effect, the radionuclide needs to decay within the DNA molecule either incorporated into the backbone or placed in between the strands [4]. Hence, in order to take advantage of the Auger effect it is of fundamental importance that the radionuclide is delivered to the

nucleus and, furthermore, highly desirable that it can be guided to a position in very close proximity to DNA (Fig. 1).

During the last few years several approaches have been suggested to deliver Auger-emitting radionuclides specifically to tumor-cell nuclei. Focus has been on delivery of ^{111}In utilizing nuclear-localization sequences (NLS) [5-8]. Proteins or peptides tagged with NLS can be transported form cytoplasmic compartments to the nucleus and if such proteins or peptides are radiolabeled and targeted to tumor markers a successful delivery of radionuclides to tumor-cell nuclei can potentially be achieved. Encouraging results from studies employing NLS-tagged radiolabeled peptides and proteins verify an increased therapeutic effect compared to that obtained with the corresponding radiolabeled targeting agent lacking NLS [6]. A possible drawback of the NLS-based strategy is the absence of DNA targeting. To take advantage of the so-called Auger effect close proximity to DNA is required, as described above. Utilization of NLS may well induce redistribution of radionuclides from cytoplasm to nucleus but not necessarily to DNA. Positive charge of NLSs could theoretically enhance association to DNA but this still remains to be demonstrated. Recently, an alternative approach for nuclear delivery of Auger emitting radioiodine based on the use of a polymer bound DNA-intercalating ellipticine derivative was suggested by Sedlacek et al. [9]. The concept opens up for DNA-delivery of radionuclides, but depends for its success on a rather complex sequence of events, including accumulation of the polymer-conjugate in tumor tissue due to the enhanced permeation and retention (EPR) effect, non-specific endocytosis of the conjugate, as well as, pH-dependent cleavage of the same to release the radiolabeled intercalator. Moreover, from the reported data [9] it is clear that the specific

radioactivity of the ellipticine derivative has to be significantly improved in order to achieve therapeutic effect.

In contrast to the strategy suggested by Sedlacek et al. [9], the Nuclisome concept enables specific targeting of tumor cells and permits efficient and direct delivery of large amounts of radiolabeled compounds to the cells. In the Nuclisome approach proximity to DNA is achieved by utilizing a specially designed intercalating compound that binds strongly to DNA. After radiolabelling, the DNA-binding compound is loaded into tumor-targeted liposomes. The liposomes deliver, in a first step, the compound to the tumor cells, and in a second step, the compound guides and delivers the radionuclide into DNA (Fig. 2). The potential of our concept based on targeting nuclide-filled liposomes, termed Nuclisome-particles, has recently been evaluated in a number of cell and animal models. Some key results from these investigations, in which we have chosen to use ^{125}I as Auger-electron emitting radionuclide, will be described in the following sections.

Compound development

In order for the two-step targeting approach to be successful, the radionuclide-labeled compound must fulfill a number of important criteria. Obviously, the compound must display high affinity for DNA and it is important that it can be encapsulated and retained in liposomes. Further, it is crucial that the compound after cellular uptake of the liposomes is effectively released into the cytoplasm. Finally, it is essential that the compound subsequent to cytoplasm release finds it way to and accumulates in the cell nucleus.

In our search for suitable compound candidates to bind nuclides to tumor-cell DNA we have focused our attention on low-molecular weight molecules with established DNA-binding capacity. Initially, several attempts were made using boronated phenanthridinium and acridine analogues [10-15]. These were primarily intended for boron neutron capture therapy but the compounds possessed properties that allowed them to serve as precursors for radiolabeling as well. Although several of the analogues could be satisfactorily loaded into targeted liposomes, none of the investigated compounds displayed the required ability to reach and accumulate in the nucleus of tumor cells. Further development and characterization of these derivatives were therefore abandoned. With the aim to identify an alternative class of molecules with capability to facilitate DNA-delivery of the Auger-electron emitting radionuclides, we turned our attention to the anthracyclines.

Many anthracyclines can be successfully encapsulated in liposomes, and, in particular, liposomal doxorubicin and daunorubicin have been frequently used in studies aiming at both passive [16,17] and active [18] targeting of tumor cells. Inspired by these studies, we set out to investigate a series of specially designed and synthesized amino-benzyl derivatives of daunorubicin [19]. The derivatives synthesized were mainly substituted at the N-3' position in the sugar moiety or in C-13 position in the core structure. Based on results of initial tests, three compounds, Comp1-3, were selected for further analysis and characterization [20].

When tested in iodinated form, all three compounds were found to bind to calf thymus DNA with equal or stronger affinity than that found for the mother compound daunorubicin. A comparison of the determined binding constants and exclusion

parameters indicated, however, that Comp1 and Comp2 interacted more favorably with DNA than did Comp3. Moreover, as probed by laser confocal microscopy, Comp3 showed little, or no, tendency to accumulate in the nucleus of living cells. A similar lack of nuclear staining was observed for cells treated with Comp2. For Comp1, on the other hand, the confocal microscopy images exhibited a fluorescence pattern indicative of nuclear staining. In line with this, cells treated with ^{125}I-labeled Comp1 displayed clear nuclear association of the radioactive decay when analyzed by means of autoradiography. Taken together, Comp1 demonstrated the most promising properties for ^{125}I-labeling and subsequent use as active component in Nuclisome-particles (Tab. 1). ^{125}I-Comp1 was further characterized in terms of its ability to cause double stranded breaks (DBS) in extracted chromosomal DNA. Based on DNA-fragmentation analyses, using pulsed-field gel electrophoresis, it was possible to determine that ^{125}I-Comp1 renders about 0.4 DSB/decay. DNA-fragmentation of this magnitude can be expected only when the radionuclide is positioned close to DNA. For comparison, ^{125}I-labeled DNA bases, for which maximum Auger effect is achieved, have been shown to generate about 0.8 DBS/decay under comparable conditions [20]. When evaluating the extent of DNA-fragmentation obtained with ^{125}I-Comp1 it should be remembered that the efficacy with which an Auger decay causes strand breaks decreases with its distance from the central DNA-axis. It is in this context interesting to note that about half the DSB/decay found for ^{125}I-labeled DNA bases can be expected upon positioning ^{125}I roughly one nanometer further away from the central DNA-axis [21]. The observed reduction from 0.8 to 0.4 DSB/decay in going from ^{125}I-labeled DNA bases to ^{125}I-Comp1 is thus in line with the predictions.

Target specificity

A large number of potential targets, including carcinoembryonic antigen (CEA) [22], folic acid receptor [23], EGFR [12], HER2 [24], CD19 [25] and somatostatin receptors (SSTRs) [26] have been suggested for targeting liposomes. For the Nuclisome-particles we have so far chosen to focus on targeting against members of the EGF-receptor family. The EGF-receptor family consists of the four transmembrane receptors EGFR, HER2, HER3 and HER4 [27]. In normal cells, the receptors are responsible for proliferation and differentiation. However, disturbances in their regulation and signaling pathways can result in malignancies [28,29]. Many tumor cells are known to express one or several of the EGF family receptors to a degree that far exceeds that found in normal cells. Over-expression of EGFR is found in, for example, lung, breast, colon, prostrate, as well as squamous cell cancer of the head and neck [28] and overexpression of HER2 is found in, for example, breast, ovarian, and stomach cancers [27]. This over-expression is mainly due to gene amplification and is often associated with tumor progression and poor prognosis [27,30]. Due to the common over-expression and the link to poor prognosis, we have in our studies performed to date selected EGFR and HER2 as the primary targets for our Nuclisome-particles.

For targeting against EGFR we have employed the natural ligand EGF as targeting agent, whereas for targeting against HER2 a single-chains fragment (F5) [31] have been used. In both cases the targeting agents have been conjugated to the distal end of polyethylene glycol (PEG) in PEG-lipids. The EGF-PEG-lipid and F5-PEG-lipid conjugates have subsequently been incorporated into liposomes composed of DSPC, cholesterol, and DSPE-PEG2000 (see e.g. [32,33]). Finally, the liposomes have been loaded with iodinated

Comp1 using the pH-gradient-driven loading protocol which was first suggested by Mayer et al. [34].

The receptor specificity of the EGFR- and HER2-targeted Nuclisome-particles has been investigated and verified in two separate *in-vitro* studies. By use of cellular assays that permitted quantification of the receptor-mediated uptake of the particles, we could confirm that the Nuclisome-particles are indeed taken up by the cultured cells through interaction with EGFR and HER2, respectively [32,33]. Importantly, the above studies showed that when receptors were blocked with an excess amount of free ligand, or liposomes were administered without targeting agent, the tumor-cell uptake was clearly suppressed.

Receptor specificity was further manifested in an *ex vivo* human vasculature system (Chandler loop) [32]. In the set up, fresh human blood from healthy donor was mixed with cultured human tumor cells, mimicking circulating tumor cells. EGFR-targeted Nuclisome-particles were added to the loop and the target specificity was analyzed a few hours after administration. All three parts of the Nuclisome-particles, the targeting agent, the liposome, and the drug, exhibited a substantial uptake in EGFR-expressing tumor cells while the uptake in white blood cells was minor. The uptake of ^{125}I-Comp1 in tumor cells was almost 35-times higher than that in white blood cells, which leads to a significant difference in absorbed dose. The results from *ex vivo* experiments thus clearly demonstrate the potential of Nuclisome-particles to deliver a DNA-binding Auger-electron emitter to circulating tumor cells.

Therapeutic effect *in vitro*

The *in-vitro* evaluation of the Nuclisome-particles has included confocal microscopy and autoradiography studies to verify that the radiolabeled drug, after cellular uptake of the particles, is set free in the cytoplasm and able to translocate to the cell nucleus [32,33]. These studies have been followed up by investigations designed to evaluate the therapeutic effect of the particles. Results of recently reported cell studies indicate a remarkably high tumor-cell killing efficiency of EGFR-targeted Nuclisome-particles as compared to that of EGFR-targeted liposomes loaded with ^{127}I-labeled Comp1 or the conventional anthracycline doxorubicin [32]. Under the experimental conditions employed non-targeting liposomes loaded with ^{127}I-Comp1 or doxorubicin had no discernible inhibitory effect on the growth of cultured tumor cells. It can be noted that non-targeted liposomes containing doxorubicin are presently clinically used for treatment of Kaposi's sarcoma and breast cancer. The commercially available formulations employ either PEG-stabilized (Caelyx/Doxil) or non-stabilized (Myocet) liposomes.

An increased efficacy of doxorubicin-loaded liposomes could in our studies be seen by adding EGF as targeting agent, confirming the previously reported efficacy enhancement achieved with targeting liposomes [35]. However, EGFR-targeted Nuclisome-particles loaded with the ^{125}I-labeled Comp1 were up to five orders of magnitude more effective than EGFR-targeted liposomes loaded with doxorubicin (Tab. 2). Importantly, upon replacing ^{125}I-Comp1 in the targeting Nuclisome-particles with ^{127}I-Comp1 the growth inhibitory effect was completely lost. It is thus evident that the dramatic effect of the ^{125}I-Comp1- loaded targeting Nuclisome-particles derives from ^{125}I solely. Keeping in

mind that autoradiography of cells incubated with EGFR-targeted Nuclisome-particles showed that radioactive decays were co-localized with the nuclei of tumor cells [32], it appears plausible that ^{125}I causes an Auger effect on tumor-cell DNA.

A similar high tumor-specific cell killing potential has been established also for HER2-targeted Nuclisome-particles [33]. Further, data collected verified a dose-response correlation compatible with that expected following high linear energy transfer irradiation. This finding further supports the suggestion of Nuclisome-particles being able to provide a biological Auger effect.

Therapeutic effect *in vivo*

The *in vivo* therapeutic effect of Nuclisome-particles was recently demonstrated in a dose-escalating efficacy study [36]. Ten million HER2-expressing human ovarian cancer cells were injected i.p. in nude mice followed by i.p. administration of HER2-targeted Nuclisome-particles. Different groups received the same amount of Nuclisome-particles but with varying specific radioactivity. The results revealed a clear correlation between administered radioactivity and survival of the mice. With HER2-targeting Nuclisome-particles a significant increase in survival, as compared to that of untreated control mice, could be seen already at an administered radioactivity corresponding to 0.1 MBq/mouse (Fig. 3). Further increase in administered radioactivity lead to prolonged survival. At the highest radioactivity administered, 2 MBq/mice, 70% of the mice survived the study and the majority of these were tumor-free. Dose-dependent survival was not observed for mice treated with Nuclisome-particles lacking HER2-targeting ability.

Notably, neither macroscopic nor microscopic radiotoxic effects in normal tissues were observed. Dosimetric calculations based on previous biodistribution data, and, assuming non-functional two-step targeting in organs not expressing HER2, suggested that mean mean absorbed doses to normal tissues were low (Tab. 3). The organ accumulating most Nuclisome-particles, spleen, received according to the calculations merely 0.3 Gy during treatment with 2 MBq/mouse. Since the accumulation of ^{125}I in spleen was higher than in tumor cells, this finding constitutes a further, indirect, evidence to support that an Auger effect was achieved in receptor-targeted tumor cells. More specifically, in the absence of an Auger effect an absorbed dose of less than 0.3 Gy in tumor cells would not give the dramatic tumor cell eradication and prolonged survival seen in our study.

Possible clinical applications

The high specificity and remarkable potency make Nuclisome-particles interesting for several clinical indications. Results obtained to date suggest that treatment with Nuclisome-particles has the potential to become an effective therapy against metastasizing cancer cells. An important target could be breast cancer and the large amount of activity delivered per each Nuclisome-receptor interaction opens up for the possibility of treating single tumor cells circulating in the blood. Further, spread tumor cells are most suitable as targets since liposomes have limited penetration in solid tumors. Ovarian cancer with spread tumor cells in peritoneum can be foreseen as another suitable application, as demonstrated in the recent animal study. Moreover, by exchanging the targeting agent Nuclisome-particles could be directed against tumor cells originating from, e.g., prostate and colon cancer, or tailored for treatment of endocrine tumors.

Noteworthy, the choice of Auger-electron emitting nuclides as active components in the Nuclisome-particles circumvents two of the major drawbacks faced by current protocols for clinical radionuclide therapy. Due to the very local effect, limited to only targeted cells, normal tissue and dose-limiting organs, such as bone marrow, are spared from crossfire from the nuclide. Further, due to the short range of the Auger electrons, the need for special nuclear medicine facilities and patient isolation could be avoided. An example may serve to illustrate the possible benefits of utilizing Auger-electron emitting nuclides for tumor therapy. A crude estimate suggests that the amount of circulating single tumor cells or micro-metastases in a patient with disseminated disease corresponds to about one gram. If roughly a thousand decays of ^{125}I could be generated in the DNA of each cancer cell, the patient would stand a very good chance of being cured. The total radioactivity involved in such a situation corresponds to about 0.1 MBq, which when injected in the body, without attaching to cellular DNA, corresponds to about 5 mSv for the treated patient [4]. This is approximately equal to the yearly background dose received by the average Swede from ionizing radiation.

Acknowledgements

Finiancial support from Swedish Cancer Society, the Swedish Research Council and Stiftelsen Olle Engkvist Byggmästare is gratefully acknowledged.

References

1. Fenske DB, Chonn A, Cullis PR (2008) Liposomal nanomedicines: an emerging field. Toxicologic pathology 36 (1):21-29

2. Sapra P, Allen TM (2003) Ligand-targeted liposomal anticancer drugs. Prog Lipid Res 42 (5):439-462

3. Elmroth K, Stenerlow B (2005) DNA-incorporated 125I induces more than one double-strand break per decay in mammalian cells. Radiat Res 163 (4):369-373

4. Lundqvist H, Stenerlöw B, Gedda L (2008) The Auger effect in molecular targeting therapy. In: Stigbrand T AGaCJ (ed) Targeted radionuclide tumor therapy. Springer Sciences + Business Media B.V, pp 197-216

5. Chen P, Wang J, Hope K, Jin L, Dick J, Cameron R, Brandwein J, Minden M, Reilly RM (2006) Nuclear localizing sequences promote nuclear translocation and enhance the radiotoxicity of the anti-CD33 monoclonal antibody HuM195 labeled with 111In in human myeloid leukemia cells. J Nucl Med 47 (5):827-836

6. Costantini DL, Chan C, Cai Z, Vallis KA, Reilly RM (2007) (111)In-labeled trastuzumab (Herceptin) modified with nuclear localization sequences (NLS): an Auger electron-emitting radiotherapeutic agent for HER2/neu-amplified breast cancer. J Nucl Med 48 (8):1357-1368

7. Costantini DL, Villani DF, Vallis KA, Reilly RM (2010) Methotrexate, paclitaxel, and doxorubicin radiosensitize HER2-amplified human breast cancer cells to the Auger electron-emitting radiotherapeutic agent (111)In-NLS-trastuzumab. J Nucl Med 51 (3):477-483

8. Ginj M, Hinni K, Tschumi S, Schulz S, Maecke HR (2005) Trifunctional somatostatin-based derivatives designed for targeted radiotherapy using auger electron emitters. J Nucl Med 46 (12):2097-2103

9. Sedlacek O, Hruby M, Studenovsky M, Kucka J, Vetvicka D, Kovar L, Rihova B, Ulbrich K (2011) Ellipticine-aimed polymer-conjugated auger electron emitter: multistage organelle targeting approach. Bioconjug Chem 22 (6):1194-1201

10. Bohl Kullberg E, Carlsson J, Edwards K, Capala J, Sjoberg S, Gedda L (2003) Introductory experiments on ligand liposomes as delivery agents for boron neutron capture therapy. Int J Oncol 23 (2):461-467

11. Gedda L, Silvander M, Sjoberg S, Tjarks W, Carlsson J (1997) Cytotoxicity and subcellular localization of boronated phenanthridinium analogues. Anticancer Drug Des 12 (8):671-685

12. Kullberg E, Bergstrand N, Carlsson J, Edwards K, Johnsson M, Sjoberg S, Gedda L (2002) Development of EGF-Conjugated Liposomes for Targeted Delivery of Boronated DNA-Binding Agents. Bioconjug Chem 13 (4):737-743.

13. Kullberg EB, Nestor M, Gedda L (2003) Tumor-cell targeted epiderimal growth factor liposomes loaded with boronated acridine: uptake and processing. Pharm Res 20 (2):229-236

14. Kullberg EB, Stenerlow B, Ghirmai S, Lundqvist H, Malmstrom PU, Orlova A, Tolmachev V, Gedda L (2005) An aminoacridine derivative for radionuclide therapy: DNA binding properties studied in a novel cell-free in vitro assay. Int J Oncol 27 (5):1355-1360

15. Wei Q, Kullberg EB, Gedda L (2003) Trastuzumab-conjugated boron-containing liposomes for tumor-cell targeting; development and cellular studies. Int J Oncol 23 (4):1159-1165

16. Gabizon AA (1992) Selective tumor localization and improved therapeutic index of anthracyclines encapsulated in long-circulating liposomes. Cancer Res 52 (4):891-896.

17. Papahadjopoulos D, Allen TM, Gabizon A, Mayhew E, Matthay K, Huang SK, Lee KD, Woodle MC, Lasic DD, Redemann C, et al. (1991) Sterically stabilized liposomes: improvements in pharmacokinetics and antitumor therapeutic efficacy. Proc Natl Acad Sci U S A 88 (24):11460-11464.

18. Allen TM, Cullis PR (2004) Drug delivery systems: entering the mainstream. Science 303 (5665):1818-1822

19. Ghirmai S, Mume E, Tolmachev V, Sjoberg S (2005) Synthesis and radioiodination of some daunorubicin and doxorubicin derivatives. Carbohydr Res 340 (1):15-24

20. Ickenstein LM, Edwards K, Sjoberg S, Carlsson J, Gedda L (2006) A novel 125I-labeled daunorubicin derivative for radionuclide-based cancer therapy. Nucl Med Biol 33 (6):773-783

21. Chen K, Adelstein SJ, Kassis AI (2004) Molecular simulation of ligand-binding with DNA: implications for 125I-labeled pharmaceutical design. Int J Radiat Biol 80 (11-12):921-926

22. Yanagie H, Tomita T, Kobayashi H, Fujii Y, Takahashi T, Hasumi K, Nariuchi H, Sekiguchi M (1991) Application of boronated anti-CEA immunoliposome to tumour cell growth inhibition in in vitro boron neutron capture therapy model. Br J Cancer 63 (4):522-526

23. Pan XQ, Wang H, Shukla S, Sekido M, Adams DM, Tjarks W, Barth RF, Lee RJ (2002) Boron-containing folate receptor-targeted liposomes as potential delivery agents for neutron capture therapy. Bioconjug Chem 13 (3):435-442

24. Park JW, Hong K, Carter P, Asgari H, Guo LY, Keller GA, Wirth C, Shalaby R, Kotts C, Wood WI, et al. (1995) Development of anti-p185HER2 immunoliposomes for cancer therapy. Proc Natl Acad Sci U S A 92 (5):1327-1331.

25. Lopes de Menezes DE, Pilarski LM, Allen TM (1998) In vitro and in vivo targeting of immunoliposomal doxorubicin to human B-cell lymphoma. Cancer Res 58 (15):3320-3330

26. Sun M, Wang Y, Shen J, Xiao Y, Su Z, Ping Q (2010) Octreotide-modification enhances the delivery and targeting of doxorubicin-loaded liposomes to somatostatin receptors expressing tumor in vitro and in vivo. Nanotechnology 21 (47):475101

27. Mendelsohn J, Baselga J (2000) The EGF receptor family as targets for cancer therapy. Oncogene 19 (56):6550-6565

28. El-Rayes BF, LoRusso PM (2004) Targeting the epidermal growth factor receptor. Br J Cancer 91 (3):418-424

29. Ranson M, Sliwkowski MX (2002) Perspectives on anti-HER monoclonal antibodies. Oncology 63 Suppl 1:17-24

30. Spano JP, Fagard R, Soria JC, Rixe O, Khayat D, Milano G (2005) Epidermal growth factor receptor signaling in colorectal cancer: preclinical data and therapeutic perspectives. Ann Oncol 16 (2):189-194

31. Nielsen UB, Kirpotin DB, Pickering EM, Hong K, Park JW, Refaat Shalaby M, Shao Y, Benz CC, Marks JD (2002) Therapeutic efficacy of anti-ErbB2 immunoliposomes targeted by a phage antibody selected for cellular endocytosis. Biochim Biophys Acta 1591 (1-3):109-118

32. Fondell A, Edwards K, Ickenstein LM, Sjoberg S, Carlsson J, Gedda L (2010) Nuclisome: a novel concept for radionuclide therapy using targeting liposomes. Eur J Nucl Med Mol Imaging 37 (1):114-123

33. Fondell A, Edwards K, Unga J, Kullberg E, Park JW, Gedda L (2011) In vitro evaluation and biodistribution of HER2-targeted liposomes loaded with an (125)I-labelled DNA-intercalator. J Drug Target. 19(9):846-55

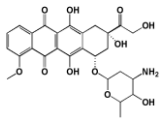
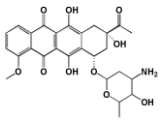
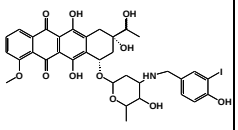
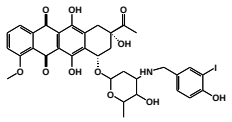
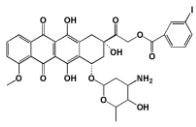
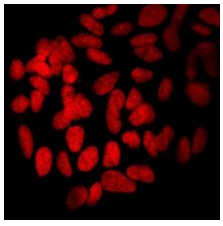
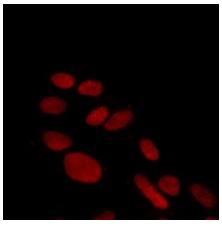
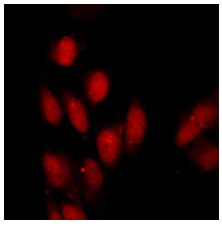
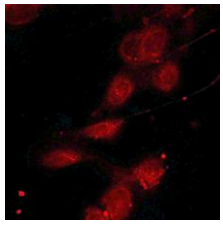
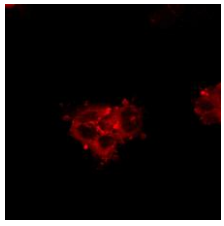
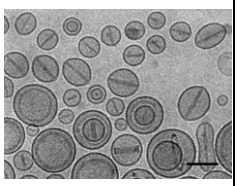
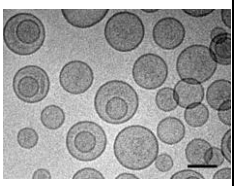
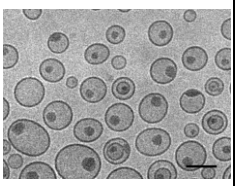
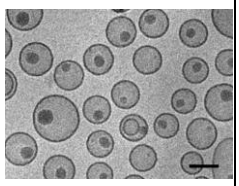
34. Mayer LD, Bally MB, Cullis PR (1986) Uptake of adriamycin into large unilamellar vesicles in response to a pH gradient. Biochim Biophys Acta 857 (1):123-126

35. Park JW, Hong K, Kirpotin DB, Colbern G, Shalaby R, Baselga J, Shao Y, Nielsen UB, Marks JD, Moore D, Papahadjopoulos D, Benz CC (2002) Anti-HER2 Immunoliposomes: Enhanced Efficacy Attributable to Targeted Delivery. Clin Cancer Res 8 (4):1172-1181.

36. Gedda L, Fondell A, Lundqvist H, Park JW, Edwards K (2011) Experimental radionuclide therapy of HER2-expressing xenografts using two-step targeting Nuclisome-particles. Journal of Nuclear Medicine, Accepted.

37. Kassis AI, Adelstein SJ (2005) Radiobiologic principles in radionuclide therapy. J Nucl Med 46 Suppl 1:4S-12S

TABLE 1. Properties of compounds used with the Nuclisome concept.

	Doxorubicin	**Daunorubicin**	**I-Comp1**	**I-Comp2**	**I-Comp3**
					
MW	543.5 (HCl salt: 580)	527.5 (HCl salt: 564)	^{127}I: 761 ^{125}I: 759	^{127}I: 759 ^{125}I: 757	^{127}I: 774
Synthesis	Commercial	Commercial	Girmai et al. [19]	Girmai et al. [19]	Girmai et al. [19]
DNA-binding constants[a] **Ki (x 10^6 M^{-1})**	3.2 (±1.1)	0.7 (±0.1)	3.2 (±0.4)	3.0 (±1.5)	1.3 (±0.2)
DNA-exclusion parameters[a] (n)	3.9 (±0.4)	3.6 (±0.3)	3.6 (±0.6)	3.7 (±0.7)	7.8 (±0.4)
Uptake in living cells *per se*[a, b]	Nuclear staining 	Nuclear staining 	Nuclear staining 	Perinuclear staining 	Perinuclear staining 
Cryo-TEM images showing drug-citrate precipitate formed inside liposomes (size bar = 100 nm)	Fibrous bundles[b, c] 	Diffuse crystals 	Globular precipitate[b, c] 	Globular precipitate 	

[a] Ickenstein et al. [20]

[b] Reprinted with permission from the publisher.

[c] Fondell et al. [32]

TABLE 2. Survival of cultured human tumor cells treated with HER2-targeting and non-targeting liposomes. Data compiled from Fondell et al. [32].

Treatment	**Survival (%)**
HEPES buffered saline (Control)	100
Targeting-liposome DOX	10
Targeting-liposome ^{127}I-Comp1	100
Targeting-liposome ^{125}I-Comp1 (Nuclisome-particle)	0.0001
Non-targeting liposome ^{125}I-Comp1	10

TABLE 3. Estimated mean absorbed doses to normal organs as a function of administered radioactivity. Data compiled from Gedda et al. [36].

	Mean absorbed dose per administered radioactivity (mGy/MBq)	
	Non-targeting	**HER2-targeting**
Blood	18.7	1.8
Spleen	36.6	150.0
Liver	15.4	27.1
Kidneys	15.0	9.4

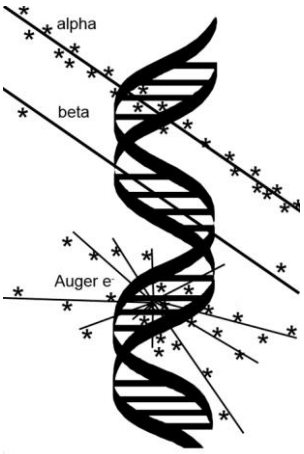
NOTE: Mean doses are presented under the assumption of non-functional two-step targeting (no HER2 expression) and that the distribution of activity in the therapy study follows the biodistribution of HER2-targeting and non-targeting liposomes presented in Fondell et al. [33].

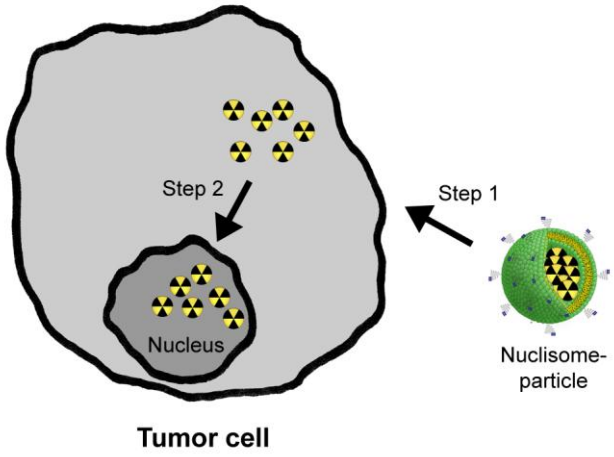
Figure legends

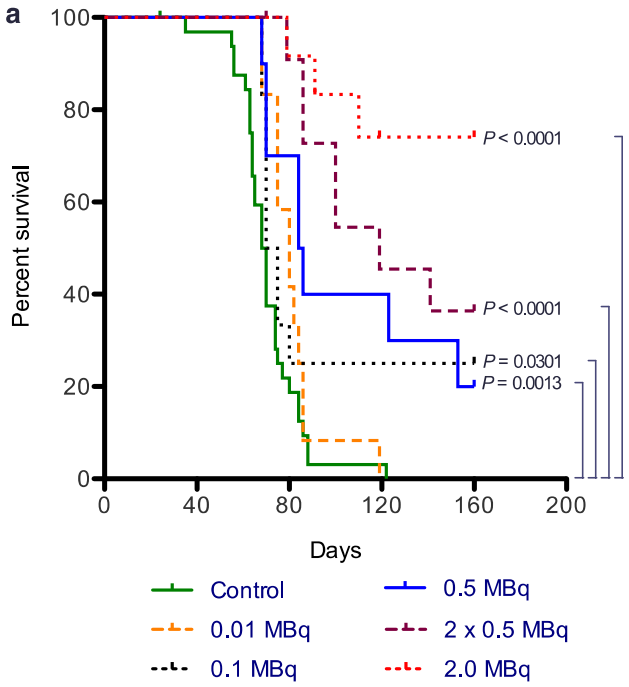
Fig. 1 Schematic illustration (adapted from [37]) of local ionizations (*) along tracks of an alpha particle, a beta particle and electrons from an Auger-electron emitter. A beta particle is sparsely ionizing along its track while an alpha particle is densely ionizing. The cluster of electrons released by an Auger-electron emitting radionuclide produce local dense ionizations

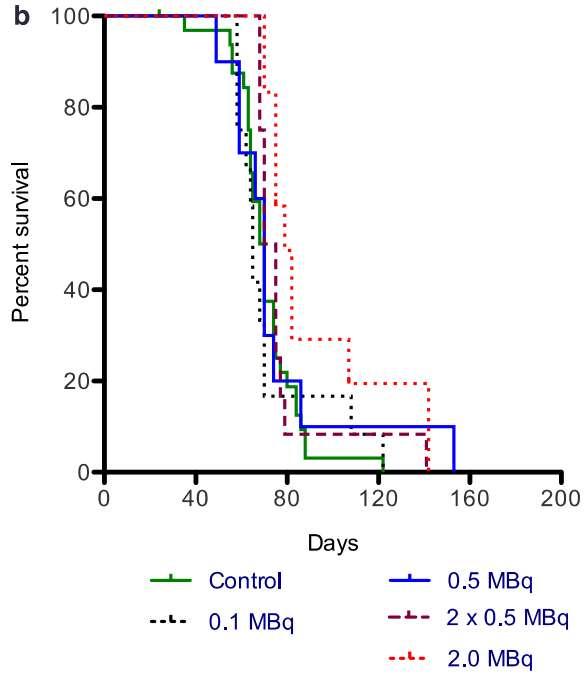
Fig. 2 Principle of the Nuclisome two-step targeting concept. In step 1 Auger-electron emitting radionuclides are delivered to the tumor cell by means of liposomes targeted to structures present on the cell surface. In step 2 the radionuclides are guided to the cell nucleus and exert their DNA damaging effect

Fig. 3 Kaplan-Meier plot showing the survival of mice with HER2-expressing i.p. xenografts. Control mice were treated with HEPES buffered saline (n=33). A: Mice treated with 0.01 MBq/mouse (n=12), 0.1 MBq/mouse (n=12), 0.5 MBq/mouse (n=10), 2 × 0.5 MBq/mouse (n=12) and 2 MBq/mouse (n=12) of HER2-targeting liposomes (Nuclisome-particles). B: Mice treated with 0.1 MBq/mouse (n=12), 0.5 MBq/mouse (n=10), 2 × 0.5 MBq/mouse (n=12) and 2 MBq/mouse of non-targeting liposomes (n=12). Data compiled from Gedda et al. [36]


alpha
beta
Auger e-


Step 2
Step 1
Nucleus
Nuclisome-
particle
Tumor cell


a
100
80
60
40
20
0
Percent survival
P < 0.0001
P < 0.0001
P = 0.0301
P = 0.0013
0
40
80
120
160
200
Days
Control
0.01 MBq
0.1 MBq
0.5 MBq
2 x 0.5 MBq
2.0 MBq


b
100
80
60
40
20
0
Percent survival
0
40
80
120
160
200
Days
Control
0.1 MBq
0.5 MBq
2 x 0.5 MBq
2.0 MBq