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Searching for Synergy

Radiosensitization of ^{177}Lu-DOTATATE

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ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2022

ISSN 1651-6206
ISBN 978-91-513-1358-0
URN urn:nbn:se:uu:diva-459961

Dissertation presented at Uppsala University to be publicly examined in Rudbecksalen, Rudbecklaboratoriet, Dag Hammarskjölds väg 20, Uppsala, Friday, 4 February 2022 at 09:15 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in English. Faculty examiner: Julie Nonnekens (Erasmus University Medical Center, Molecular Genetics and Radiology & Nuclear Medicine).

Abstract

Lundsten, S. 2022. Searching for Synergy. Radiosensitization of ^{177}Lu-DOTATATE. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 1797. 58 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-513-1358-0.

Cancers presents a major health challenge, and there is a pressing need to develop new therapeutic strategies. Surgery, chemotherapy and radiation are the most commonly used treatments for cancer today. Radiation can be given as targeted radionuclide therapy (TRT), i.e., systemic administration of a radiolabeled cancer-targeting molecule. This is especially suitable for inoperable and disseminated tumors.

^{177}Lu-DOTATATE, a TRT directed against the somatostatin receptors (SSTRs), was recently approved for therapy of a subset of neuroendocrine tumors (NETs). Although it has prolonged the life of NET patients, complete remission is seldom achieved. Consequently, to increase the efficacy of the treatment, this thesis aimed to assess potential radiosensitizing strategies for ^{177}Lu-DOTATATE. The two radiosensitization targets in focus were HSP90, a chaperone protein with numerous oncogenic client proteins, and p53, a central regulator of DNA damage.

In **papers I and II**, we investigated the HSP90-inhibitor Onalespib, as a treatment for NETs, and as a potential radiosensitizer. The drugs were assessed *in vitro* and *in vivo*. We concluded that Onalespib reduced NET cell growth and acted synergistically with ^{177}Lu-DOTATATE. Inhibition of EGFR, a HSP90 client protein, was suggested as a mediator of the observed synergy. Furthermore, the combination had a favorable toxicity profile.

In **paper III**, we assessed the novel stapled peptide VIP116, which inhibits the p53 repressors MDM2 and MDM4, as a potentiator of ^{177}Lu-DOTATATE in wildtype p53 neuroblastoma cells. Combination therapy exhibited growth-inhibitory effects, with resulting additive or synergistic effects. The treatment-mediated effects on p53 signaling were characterized, revealing a possible involvement of V-myc myelocytomatosis viral oncogene homolog, neuroblastoma derived (MYCN), a prognostic marker for poor survival in neuroblastoma.

In **paper IV**, we aimed to improve targeted delivery of VIP116, with the use of lipid bilayer disks (lipodisks). VIP116 was successfully loaded onto epidermal growth factor receptor (EGFR)-targeting lipodisks, leading to specific delivery and reduction of viability of EGFR expressing tumor cells. The study provided a proof-of-concept for utilizing lipodisks as a drug delivery system for p53-stabilizing peptides.

In conclusion, we have investigated, and found, suitable candidates for potentiating ^{177}Lu-DOTATATE therapy. We have addressed the feasibility of the treatments, toxicity and targeted delivery. Moreover, the work has explored the biology of TRT. This is an area in need of more attention, as more and more radionuclide-based therapies are entering clinicals trials and reaching approval.

Keywords: Cancer, targeted radionuclide therapy, radiosensitization, p53, MDM2/MDM4 inhibition, HSP90, drug synergy, drug delivery, lipid nanoparticles, lipodisks

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ISSN 1651-6206
ISBN 978-91-513-1358-0
URN urn:nbn:se:uu:diva-459961 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-459961)

The world has only sweet moments set aside for us

From the song *Who Wants to Live Forever*, written by
Dr Brian May, astrophysicist and guitarist in Queen

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. **Lundsten, S.**, Spiegelberg, D., Stenerlow, B., & Nestor, M. (2019). The HSP90 inhibitor Onalespib potentiates ^{177}Lu-DOTATATE therapy in neuroendocrine tumor cells. *International Journal of Oncology*, 55(6):1287

II. **Lundsten, S.***, Spiegelberg, D.*, Raval, N. R. & Nestor, M. (2020). The radiosensitizer Onalespib increases complete remission in ^{177}Lu-DOTATATE-treated mice bearing neuroendocrine tumor xenografts. *European Journal of Nuclear Medicine and Molecular Imaging,* 47(4):980

III. **Lundsten, S.**, Berglund, H., Jha, P., Krona, C., Hariri, M., Nelander, S., Lane, D. P. & Nestor, M (2021). p53-mediated radiosensitization of ^{177}Lu-DOTATATE in neuroblastoma tumor spheroids. *Biomolecules,* 11(11):1695

IV. **Lundsten, S.**, Hernández, V. A., Gedda, L., Sarén, T., Brown, C. J., Lane, D. P., Edwards, K. & Nestor, M. (2020) Tumor-targeted delivery of the p53-activating peptide VIP116 with PEG-stabilized lipodisks. *Nanomaterials (Basel),* 10(4):783

*Equal contribution

Publications not included in the thesis

V. Spiegelberg, D.*, Mortensen, A. C.*, **Lundsten, S.**, Brown, C. J., Lane, D. P. & Nestor, M. (2018) The MDM2/MDMX-p53 PM2 radiosensitizes wild-type p53 tumors. *Cancer Research*, 78(17):5084

VI. Spiegelberg, D., Abramenkovs, A., Mortensen, A. C. L., **Lundsten, S.**, Nestor, M. & Stenerlöw, B. (2020) The HSP90 inhibitor Onalespib exerts synergistic anti-cancer effects when combined with radiotherapy: an in vitro and in vivo approach. *Scientific Reports*, 10(1):5923

VII. Johansson, P., Krona, C., Kundu, S., Doroszko, M., Baskaran, S., Schmidt, L., Vinel, C., Almstedt, E., Elgendy, R., Elfineh, L., Gallant, C., **Lundsten, S.**, Ferrer Gago, F. J., Hakkarainen, A., Sipilä, P., Häggblad, M., Martens, U., Lundgren, B., Frigault, M. M., Lane, D. P., Swartling, F. J., Uhrbom, L., Nestor, M., Marino, S. & Nelander S. (2020) A patient-derived cell atlas informs precision targeting of glioblastoma. *Cell Reports*, 32(2):107897

*Equal contribution

Contents

Abbreviations

AKT	Protein kinase B
ALK	Anaplastic lymphoma kinase
ATM	Ataxia-telangiectasia mutated
Bcl-2	B-cell lymphoma 2
CDK	cyclin-dependent kinase
DDR	DNA damage response
DNAPKcs	DNA-dependent protein kinase, catalytic subunit
DOTA	Dodecane tetraacetic acid
DOTATATE	DOTA-Tyr3-octreotate
DSB	Double strand break
EBRT	External beam radiotherapy
ECM	Extra-cellular matrix
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EPR	Enhanced permeability and retention
H2AX	H2A histone family member X
HDM2	See MDM2
HER2	Epidermal growth factor receptor 2
HSA	Highest single agent
HSP70	Heat shock protein 70
HSP90	Heat shock protein 90
MDM2	Mouse doubleminute 2
MDM4	Mouse double-minute 4
MDMX	See MDM4
mIBG	Iobenguane
mTOR	Mammalian target of rapamycin
MYCN	V-myc myelocytomatosis viral oncogene homolog, neuroblastoma derived
NETs	Neuroendocrine tumors
NOXA	Phorbol-12-myristate-13-acetate-induced protein 1
p21	Cyclin-dependent kinase inhibitor 1
p53	Tumor protein 53
PEG	Polyethylene glycol
PUMA	p53-upregulated mediator of apoptosis
R_{eff}	Peptide-to-lipid ratio

SSTR	Somatostatin receptor
Trk	Tropomyosin receptor kinase
TRT	Targeted radionuclide therapy
wt	Wildtype
ZIP	Zero interaction potential
γH2AX	H2AX phosphorylated at Ser139

Introduction

Cancer

The term cancer encompasses hundreds of diseases, causing over 9 million deaths each year (1). All cancers share a common denominator, i.e., chronic cell proliferation, but they are grouped by the affected organ and the cell type from which they originate. There can be large biological differences between different tumor types and even between tumors in the same subgroup (2). In an effort to reduce the complexity of this malignancy, Hanahan and Weinberg stipulated six common hallmarks for all cancers, which enable tumors to form malignant lesions: self-sufficiency in growth signals, insensitivity to anti-growth signals, tissue invasion and metastasis, limitless replicative potential, sustained angiogenesis, and evading apoptosis (3). This work was later updated with two emerging hallmarks, deregulating cellular energetics and avoiding immune destruction, as well as two characteristics: genomic instability and inflammation (2).

Precision medicine in cancer

Significant technological improvements over the last few decades have been crucial to deepen the understanding of the biology of cancer, e.g., genomics, transcriptomics and proteomics. The vast amount of data from these large-scale analyses has helped map the network of pathways involved in tumorigenesis (4).

With this increased knowledge, new therapeutic concepts have emerged. One such concept is precision medicine, where the treatment is tailored for each patient based on their genetic, molecular, and environmental background (5). This makes it possible to target specific pathways present in individual tumors, in a highly specific manner (2). By doing so, the ultimate goal is to provide the optimal treatment to meet each patient's needs.

Neuroendocrine tumors (NETs) – focus of papers I and II

NETs are tumors originating in the endocrine system, characterized by a slow growth rate and secretion of signaling molecules, e.g., insulin (6). Common sites of primary disease are the gastrointestinal tract and lungs, although they can be found throughout the body. NETs are often asymptomatic early in the

disease, which makes them difficult to diagnose. As a result, about 12–22% are metastatic at diagnosis (7).

For patients without metastasis, surgery is the main treatment option and can be used with curative intent (8). It can also be palliative, e.g., to relieve tumor-associated symptoms from the carcinoid syndrome, which occurs from increased hormone production of the tumor. Furthermore, targeted radionuclide therapy (TRT) with ^{177}Lu-dodecane tetraacetic acid (DOTA)-Tyr3-octreotate (DOTATATE) is approved for treatment of metastatic, inoperable gastroenteropancreatic NETs (9). This is discussed in further detail below. Systemic therapies with chemotherapeutics, somatostatin analogs, mammalian target of rapamycin (mTOR) inhibitors, and inhibitors of angiogenesis are also applied (6). Heat shock protein 90 (HSP90) has been suggested as a therapeutic target for NETs, after several promising preclinical studies (10-14).

Neuroblastoma – focus of paper III

Neuroblastomas originate from the sympathetic nervous system and comprise 6–10% of all childhood cancers (15, 16). However, they account for nearly 15% of all pediatric cancer deaths (17). About 50% of the neuroblastomas arise in the adrenal gland, while the rest are found alongside the spinal cord (15). The disease prognosis ranges from complete regression, even without treatment, to metastatic disease, resistance to therapy, and death (16). Worse prognosis is seen in tumors with loss of chromosome 1p or 11q, gain of chromosome 17q, and amplification of the V-myc myelocytomatosis viral oncogene homolog, neuroblastoma derived (MYCN) protooncogene (15).

Surgery and chemotherapy are the main treatment options for low- to intermediate risk tumors in need of therapy. For high-risk tumors, these are often complemented with external beam radiotherapy (EBRT) or TRT with ^{131}I-iobenguane (mIBG) as well as additional targeted therapies, e.g., against ganglioside G2 (GD2) (16, 17).

There is a need to increase the number of available therapies for high-risk neuroblastoma. Promising approaches include TRT with ^{177}Lu-DOTATATE, targeted therapies against anaplastic lymphoma kinase (ALK), tropomyosin receptor kinase (Trk), and MYCN-related targets (16). Furthermore, tumor protein p53 (p53)-focused therapies are of interest due to the low rates of p53 mutations in neuroblastoma (18).

Radiation and cancer

Ionizing radiation and its use in nuclear medicine

A radioactive emission originates from a nuclear decay of an unstable atom, a radionuclide, as a way to release excess energy and reach an energetically stable nuclear configuration (19). The amount of excess energy present in an unstable atom is defined as its Q-value and differs between radionuclides (Table 1).

Table 1: A selection of radionuclides used for therapy and imaging of cancer (19, 20).

Nuclide	Principal radiation emitted	Q-value (keV)	Half-life	Applications
^{68}Ga	β^+	2921	1.2 hours	Imaging (PET)
^{90}Y	β^-	2280	2.7 days	Therapy, Imaging (SPECT)
^{131}I	β^-	971	8.0 days	Therapy, Imaging (SPECT)
^{177}Lu	β^-	498	6.6 days	Therapy, imaging (SPECT)
^{223}Ra	α	5979	11.4 days	Therapy
^{225}Ac	α	5935	10.0 days	Therapy

When radiation is emitted from a nuclear decay, it can interact with matter. If the emission carries enough energy to remove an electron from a molecule it interacts with, it is termed ionizing radiation. There are three main types of ionizing radiation: alpha (α), beta (β), and gamma (γ) radiation. The two former types are particle radiation, where an α particle consists of a helium nucleus, and a β particle can either be an electron (β^-), or its antiparticle, a positron (β^+). For γ radiation, the excess energy is released as an electromagnetic wave (19).

Nuclear medicine, the study and use of radiation in medicine, involves several applications and different types of radiation (Table 1). EBRT primarily utilizes γ radiation, although particle radiation, e.g., α radiation and protons, can also be applied (21). TRT have historically mainly been used with β-emitting radionuclides, although recently α emitters such as ^{223}Ra and ^{225}Ac have received increased attention (22). Molecular imaging with radionuclides entails both single photon emission tomography (SPECT) and positron emission tomography (PET). Both SPECT and PET utilize γ emission, although the latter detects the characteristic annihilation emission of 511 keV when a β^+ and a β^- particle meet (23). EBRT, TRT, and molecular imaging are discussed in more detail below.

Ionization of organic molecules, caused by direct or indirect interaction with the emitted radiation, can result in chemical and biological changes of

the molecule. When a cell is irradiated, the most detrimental damage is alterations to the DNA structure (24). The damage can vary in severity, with DSB being one of the most toxic. If the cell is unable to repair the damage, this can ultimately lead to cell death (25)

Genomic instability is central in tumorigenesis, as mutations may lead to altered signaling and an increased proliferation rate. When tumorigenesis progresses, pathways involved in sensing DNA damage and initiating repair are deregulated in order to avoid cell death (2). This leads to a vulnerability in the tumor cell since it does not have the same capability to repair DNA damage as a normal cell when being exposed to ionizing radiation (26).

Cellular response to DSBs

After a DSB occurs, a complex machinery is initiated (Figure 1). The sensing of DSB damage is poorly understood, although certain factors have been identified. An important DSB sensor is the Ataxia-Telangiectasia Mutated kinase (ATM), which is recruited to the chromatin and undergoes autophosphorylation. Phosphorylation of ATM has been proposed to cause the shift from an inactive dimer to an active monomer, which subsequently phosphorylates hundreds of downstream targets (27, 28). One substrate is the histone protein H2A histone family member X (H2AX), a well-known marker of DSB, which is involved in chromatin-based DNA damage response (DDR). H2AX is named γH2AX when phosphorylated at Ser139 (28).

DNA-dependent protein kinase, catalytic subunit (DNAPKcs), a kinase sharing many common features with ATM, is also recruited to the DSB site. Its major function is to initiate the DSB repair pathway, including phosphorylation of γH2AX (28).

A major node of DSB response is p53, a transcription factor often called “the guardian of the genome” due to its involvement in DDR (29, 30). The levels and therefore activity of p53 are tightly controlled by mouse double minute 2 (MDM2, sometimes referred to as HDM2), to which p53 is connected through an autoregulatory negative feedback loop (Figure 1) (31). In homeostatic conditions, p53 is continually produced and degraded by MDM2-mediated ubiquitination (32). ATM-mediated phosphorylation of p53 and MDM2 leads to lower binding affinity between the two, and p53 degradation is halted (28). Apart from mediating p53 degradation, MDM2 can inhibit the transcriptional activity of p53 (33). Mouse double minute 4 (MDM4, also called MDMX), a protein structurally similar to MDM2, is also able to interrupt p53 transcription. However, it lacks the E3 ligase activity of MDM2, which is responsible for the degradation of p53 (33).

p53 initiates transcription of a vast array of downstream targets involved in cell cycle arrest, senescence, and apoptosis (34). A key factor in p53-mediated cell cycle arrest is cyclin-dependent kinase inhibitor 1 (p21). It is an inhibitor of cyclin-dependent kinases (CDKs), more specifically CDK4,6/cyclin D and

CDK2/cyclin E, responsible for progression in G1/S and G2/M, respectively (35).

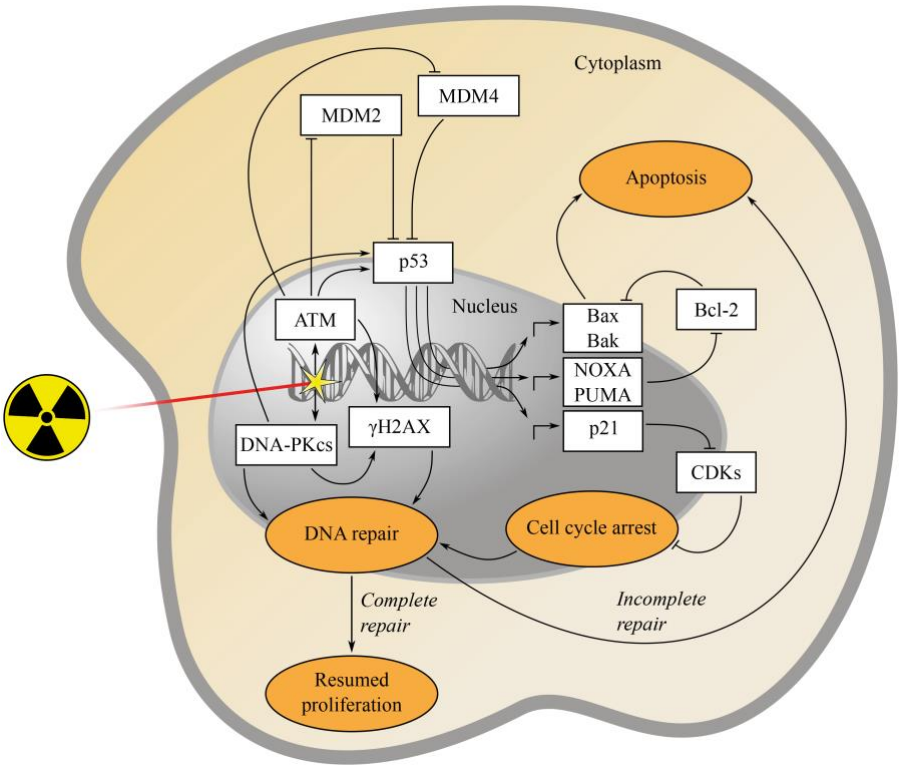

Figure 1: A simplified schematic of signaling pathways involved in the cellular response to radiation-induced DSBs. The formation of DSBs activates ATM and DNAPKcs. A repair machinery involving DNAPKcs and γH2AX is initiated, resulting in resumed proliferation (if repair is complete) or apoptosis (if repair is incomplete). ATM activates p53 with subsequent initiation of apoptosis or cell cycle arrest pathways.

p53 activation can also lead to initiation of the intrinsic apoptotic pathway (32). Upregulation of p53-upregulated mediator of apoptosis (PUMA) and Phorbol-12-myristate-13-acetate-induced (NOXA) inhibits the activity of the anti-apoptotic B-cell lymphoma (Bcl-2) protein. Furthermore, p53 upregulates pro-apoptotic Bcl-2-associated X (Bax) and Bcl-2 homologous antagonist killer (Bak). This ultimately leads to a disruption of the mitochondrial membrane and cell death (36).

There are three major models proposed to explain the choice of response pathway following p53 activation: the levels of p53, the spectrum of p53-response genes, and the availability of p53 co-factors (37). This is, however, not fully understood.

Targeting concepts

Precision medicine aims to pair the optimal treatment with each patient (5). This includes identification and development of therapies that target specific structures or pathways present on the patient's tumor. The targets can be extra- or intracellular, aiming to disrupt a pathway essential for tumor growth or deliver a payload, e.g., a toxin or radionuclide (38).

Targeted radionuclide therapy

Approximately 50% of all cancer patients are eligible for radiotherapy (39). The most common form of radiotherapy is EBRT (40). Although a powerful tool, EBRT sometimes lacks the ability to treat distant metastasis and disseminated disease (41).

TRT, which is systemically delivered, can in contrast to EBRT target lesions throughout the entire body (Figure 2). The radionuclide itself can be targeted toward certain structures, or attached to a molecule that binds to tumor cells (42). By varying the targeting molecule, the formulation can be optimized to suit the intended tumor type. The choice of radionuclide provides additional opportunities to optimize the molecule, e.g., by the type of radiation and the penetration depth of the emitted radiation (22).

Targeted therapy with radionuclides is not a new strategy. The use of ^{131}I for the treatment of thyroid disease began in the 1940s (42). It took approximately 60 years for the next TRT to enter the clinic, when ^{90}Y-labeled anti-CD20 antibody Zevalin was approved in 2002 (42). In the recent years, several other TRT compounds have reached the clinic, and many more are under development (Table 2) (22).

Table 2: A selection of TRT compounds approved for therapy and in clinical trials.

Name	Compound	Indication	Target	Status
Theracap	^{131}I	Thyroid disease	NaI symporters	Approved
Xofigo	^{223}RaCl	Bone metastasis	Bone mineralization	Approved
Lutathera	^{177}Lu-DOTATATE	Neuroendocrine tumors	Somatostatin receptor	Approved
Azedra	^{131}I-mIBG	Paragangliomas, pheochromocytomas	Adrenergic receptor	Approved
Zevalin	^{90}Y-ibritumomab	Non-Hodgkin's lymphoma	CD20	Approved
-	^{177}Lu-PSMA-617	Prostate cancer, tumor neovasculature	PSMA	Phase 3
Iomab-B	^{131}I-BC8	Bone marrow transplantation preparation	CD45	Phase 3

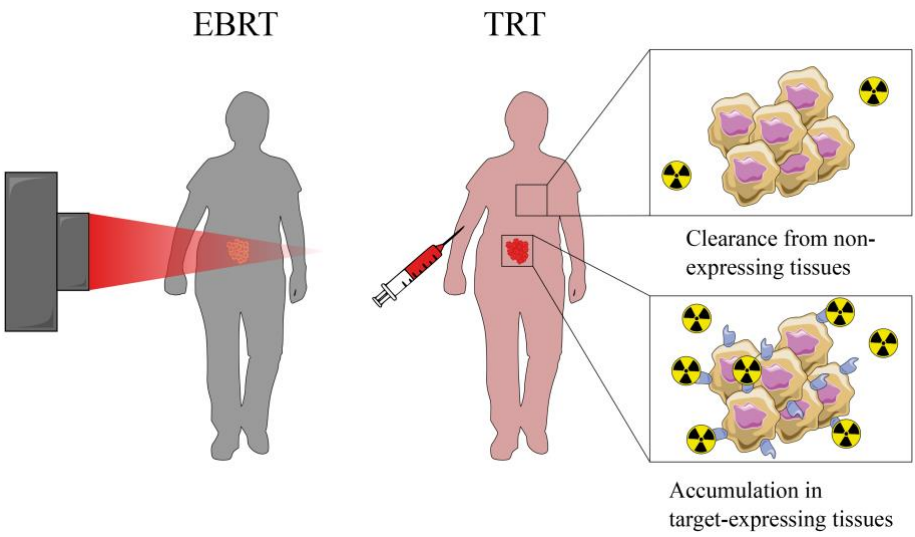

Figure 2: Radiotherapy techniques. EBRT (left) is conducted by aiming an external beam toward the tumor. Organs in front and behind the target are exposed to radiation. TRT (right) is conducted by injection of a cancer-targeting radiopharmaceutical, leading to clearance from non-expressing tissues and accumulation in target expressing tissues.

Radiopharmaceuticals can be used for both radiotherapy and imaging, e.g., with PET or SPECT. As a result, a single compound can be used for diagnosis, therapy and evaluation of therapeutic response (Figure 3). Certain radionuclides are suitable for both applications, while others are utilized for only one. In these cases, a pair of chemically similar radionuclides can be attached to the same molecule. The functional information obtained from these imaging techniques, e.g., receptor expression in tumor lesions, is often combined with anatomical imaging, such as computed tomography (CT) or magnetic resonance imaging (MRI) to correlate the tracer uptake with the anatomical position (23). This concept of combined diagnostics and therapy is often referred to as theranostics or theragnostics (43).

Molecular imaging can be used to study the *in vivo* distribution and clearance of a radiopharmaceutical. Therefore, it is possible to predict and assess potential adverse effects from the treatments, i.e., radiation exposure to healthy organs. As the radiopharmaceuticals are present in the blood during the therapy, bone-marrow is often the main dose-limiting organ. Furthermore, organs in clearance pathways, e.g., kidneys, may also receive high doses of radiation during treatment (22).

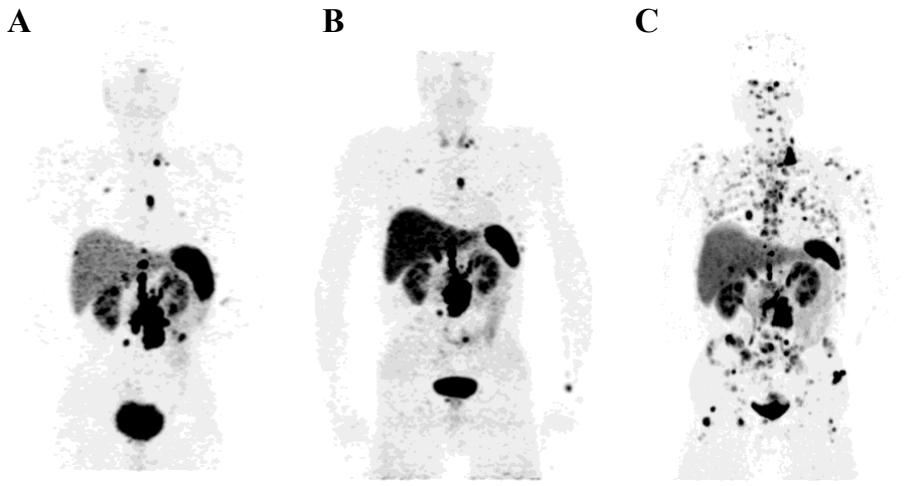

Figure 3: Theranostics with DOTATATE. ^{68}Ga-DOTATATE PET/CT in a patient with a NET in the small intestine, from a study performed by Rodrigues et. al. (44). The patient has (A) bone, lymph node and liver metastasis at baseline, (B) stable disease after one cycle of ^{177}Lu-DOTATATE therapy, and (C) progressive disease after the second round of ^{177}Lu-DOTATATE therapy. Besides the specific uptake in the tumor lesions, unspecific uptake in the liver, kidneys, spleen, and bladder are also present.

The somatostatin receptors and ^{177}Lu-DOTATATE

The somatostatin receptors (SSTRs) and their natural ligand somatostatin are expressed throughout the central nervous system and peripheral tissues. They regulate the secretion and inhibition of various hormones and enzymes, which, in turn, affects gastric emptying, smooth muscle contraction, and intestinal blood flow. There are five different SSTRs (SSTR1-5), which are part of the G protein-coupled receptor family (45). SSTR overexpression can be found in several types of tumors, including neuroendocrine tumors (NETs), neuroblastomas and medullary thyroid carcinomas (46).

Radiolabeled SSTR-binding peptides for TRT have been developed over the last 25–30 years (47), leading to the approval of ^{177}Lu-DOTATATE (Lutathera®) for therapy of well-differentiated gastroenteropancreatic NETs in 2018 (9). Targeting SSTRs for therapy of other types of tumors, including neuroblastoma, is currently being explored for diagnostic and therapeutic purposes (48-54).

^{177}Lu-DOTATATE has greatly impacted the lives of patients with NET, with improved response rates and quality of life (55, 56). Complete remission, however, is rate (55, 57-59). Therefore, strategies to improve the therapeutic response are explored. This includes the use of hepatic arterial infusion, enhancing peptide affinity, and using alternative radionuclides (60-63).

HSP90

HSP90 is a molecular chaperone, and regulates folding, activation, and degradation of its over 200 client proteins (64, 65). The human HSP90 exists as four isoforms: HSP90α, HSP90β, the 94 kDa Glucose-regulated protein (GRP94), and the HSP75/tumor necrosis factor receptor associated protein 1 (TRAP-1) (66). The protein is highly conserved throughout evolution and comprises 1–2% of a cell's total protein content, although levels can reach 4–6% when the cell is exposed to stressful conditions (65).

HSP90 functions as a homodimer and has three main regions: the N-terminal domain, responsible for ATP-binding; the middle region, which interacts with the client protein; and the C-terminal homodimerization domain (64). The HSP90 machinery (Figure 4) involves several co-chaperones, whose functions include inhibition or activation of HSP90 ATPase activity, client loading, and formation of mature HSP90 complexes (65). Binding of ATP causes the HSP90 protein to adopt its active, closed formation, leading to client protein folding and/or additional interactions between the client protein and its ligands. The cycle is completed with ADP hydrolysis, which facilitates client protein ubiquitination, and release, causing the HSP90 to transition into its inactive state (64, 66).

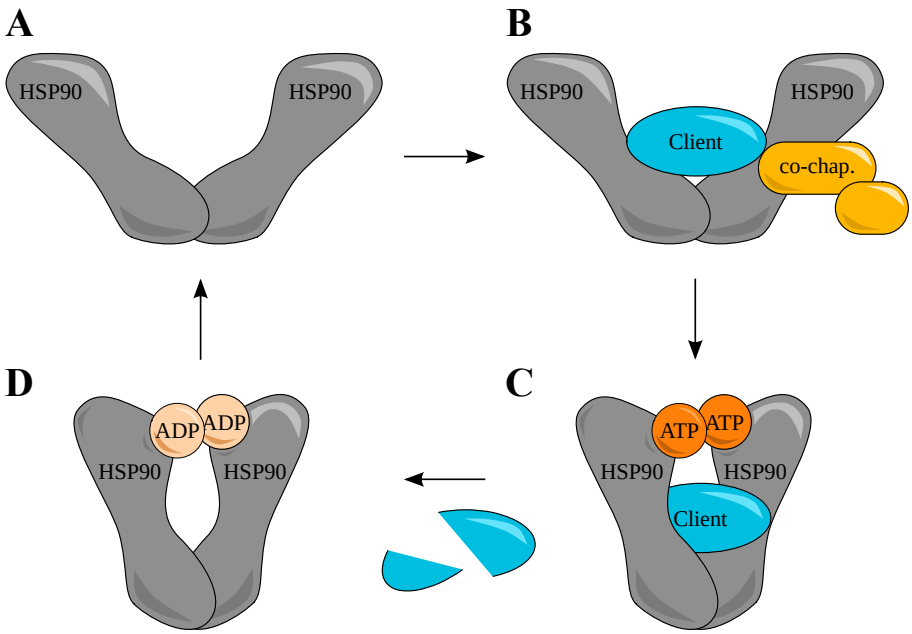

Figure 4: HSP90 function. The (A) inactive, open HSP90 binds to (B) client proteins and co-chaperones. (C) Binding of ATP leads to a closed, active complex. At this point, the client protein can be folded to the correct conformation, activated and is able to interact with ligands. Hydrolysis of ATP facilitates (D) client protein release, which can be degraded via the proteasome. APD release causes the HSP90 complex to open and return to the inactive, open conformation.

Due to the large number of oncogenic client proteins, HSP90 has been implicated as a central player in tumorigenesis and the maintenance of a malignant phenotype (64, 67, 68). Despite its essential function in normal cells, it has proven to be a promising target in cancer (67). The therapeutic window of HSP90 inhibitors has been suggested to arise from the fact that many oncogenic proteins are highly dependent on HSP90 to function properly (66).

Eighteen HSP90 inhibitors, all targeting the N-terminal ATP-binding domain, have entered clinical trials, of which Ganetespib and Tanespimycin have entered phase 3 trials (69, 70). Tanespimycin has been studied together with bortezomib in multiple myeloma, although no results have been published so far. A phase 3 trial with Ganetespib in patients with advanced lung adenocarcinoma was terminated at the interim analysis, displaying no improved effects from Ganetespib together with docetaxel compared to docetaxel alone (71). A number of trials are currently ongoing, including several novel compounds (Table 3).

Table 3: A selection of HSP90 inhibitors currently in clinical trials (70).

Compound	Combinations	Phase
TAS-116	AB122	1
XL888	Pembrolizumab, Vemurafenib, Cobimetinib	1
Zelavespib (PU-H71)		1
Gamitrinib		1
MPT0B640		1
TQB3474		1
PEN-866		1-2
Onalespib (AT13387)	AT7519, Paclitaxel, Olaparib, radiotherapy, cisplatin	1-2
Ganetespib (STA-9090)	Carboplatin, Niraparib	2[1]
Tanespimycin (KOS-953, 17-AAG)	Bortezomib	3[2]

[1]Phase 3 trial was terminated. [2]Two phase 3 trials completed; no results reported.

Although HSP90 inhibitors exhibit encouraging anti-tumor effects, none have been approved. This has been attributed to unfavorable pharmacokinetics and dose-limiting toxicities, such as hepatic and cardiac events (69). Moreover, one distinct adverse event is visual-related disorders, which in some studies have been reported to affect up to 80% of the patients (72).

The toxicities connected to HSP90 inhibition have been linked to the pan-inhibition of all members of the HSP90 family. Therefore, an inhibitor selectively targeting the cytosolic isoforms of HSP90 (HSP90α and HSP90β), named TAS-116, was recently developed (73). It was reported to have an improved toxicity profile compared to traditional HSP90 inhibitors (74, 75).

The HSP90 inhibitor chosen for the present work was Onalespib. It is a so-called second-generation HSP90 inhibitor, with a structure based on the

antifungal molecule radiciol (69). A total of 13 clinical trials, mainly phase 1, have been conducted with Onalespib, alone or as part of a combination modality (Table 3). Several of them are still ongoing. Onalespib has, either as a single agent or part of a combination therapy, exhibited a manageable toxicity profile, although with modest anti-tumor effects (76-80). Onalespib is currently being assessed in combination with radiotherapy and cisplatin as treatment of head and neck carcinoma in a phase 1 clinical study (70).

The MDM2/MDM4-p53 interaction

In approximately 50% of all cancers, the p53 protein is abrogated by mutations (32). Furthermore, p53 degradation via MDM2/MDM4 overexpression or viral factors also suppresses p53 function (32, 81). The high prevalence of p53 defects underlines the importance of the p53 pathway in tumor suppression and its potential for cancer therapy. p53 stabilization through inhibition of the MDM2-p53 interaction is currently being explored, and several MDM2 inhibitors have reached clinical trials (Table 4) (82).

Early phase trials indicate some therapeutic effects, although several compounds are reported to have dose-limiting toxicities. Common side effects include gastrointestinal events and myelosuppression, which are attributed to be on-target effects (82).

Table 4: A selection of p53-MDM2 and p53-MDM2/MDM4 inhibitors currently in clinical trials (70, 82).

Compound	Combinations	Phase
RO5503781 (RG7388, Idasanutlin)	Pegasys, Cytarabine, Daunorubicine, Venetoclax, Cyclophosphamide, Topotecan, Fludarabine, Ixazomin, Dexamethasone, Cobimetinib, Obinutuzumab, Rituximab, Atezolizumab	1-3[1]
RAIN-32 (Milademetan, DS-3032b)	Itraconazole, Posaconazole,5-azacytidine (AZA), Cytarabine, Quizartinib	1-3
AMG-232 (KRT-232	Trametinib, Decitabine, Cytarabine, Idarubicin, Radiotherapy, Dabrabenib, Cafilzomib, Lenalidomide, Dexamethasone, TL-895, Dasatinib, Nilotinib, Acalabrutinib, Ruxolitinib,	1-3
ALRN-6924	Cytarabine, Paclitaxel, Carboplatin, Pemetrexed, Topotecan	1-2
APG-115	APG-2575, Toripalimab, 5-Azacytidine (AZA), Pembrolizumab	1-2
HDM201 (Siremadlin)	Midostaurin, Trametinib, LEE011, MBG453, Venetoclax, Cytarabine, Posaconazole, Midazolam, LXS196, Ribociclib, Ruxolitinib, PDR001	1-2
BI 907828	Immune checkpoint inhibitors (BI754091, BI 754111)	1

[1]Phase 3 trial was terminated.

Although some studies are positive with regard to the clinical efficacy of MDM2 inhibition, it has been proposed that these compounds should be combined with other therapies to obtain a greater effect. Several different combination modalities are currently being explored (Table 4), including addition of chemotherapeutics as well as targeted therapies against Bcl-2, CD20, and the PI3K pathway (82).

Furthermore, as some of the resistance against MDM2 inhibitors is attributed to MDM4 activity (83, 84), dual inhibitors against MDM2 and MDM4 are being explored. One such compound is VIP116, which was used in the present work. Both VIP116 and its predecessor PM2 are synthetic peptides with a high affinity to MDM2 and MDM4 (85-88). A hydrocarbon stapling process provides the compounds with a favorable *in vivo* stability (89).

Stapled peptides are a relatively new concept in the clinic, but a phase 1 dose-escalation trial with the MDM2/MDM4-inhibiting stapled peptide ALRN-6924 was recently completed (90). The overall toxicity profile was promising where, in contrast to small-molecule MDM2 inhibitors, there were limited myelosuppressive toxicities. Additional phase 1 studies with ALRN-6924 as treatment for solid tumors (in combination with paclitaxel) or pediatric cancer (alone or in combination with cytarabine) are currently in the recruiting phase (70).

Lipid-based drug delivery systems

Drug delivery systems aim to protect and selectively deliver compounds whose efficacy may be hindered by low solubility, unfavorable pharmacokinetics, or dose-limiting toxicities. One example is lipid-based nanoparticles, which are used for delivery of chemotherapeutics, anti-fungal drugs, and oligonucleotide-based therapies and vaccines (91).

Lipid nanoparticles can be directed toward tumors by both passive and active targeting. Passive targeting includes the enhanced permeability and retention (EPR)-effect, where molecules that are large enough to avoid renal clearance (> 6–8 nm) tend to accumulate in tumor tissue due to disordered and leaky vessels (92). Moreover, the distribution and clearance of lipid nanoparticles can be optimized by the choice of lipid components and addition of polyethylene glycol (PEG), which increase the biocompatibility and prolong the elimination half-life of the structure (91).

Moreover, the use of targeting ligands on the lipid particle surface can actively direct drug delivery to target cells. So far, targeting agents directed toward the transferrin receptor and epidermal growth factor receptor 2 (HER2) have been explored for anti-cancer purposes in a clinical setting (93, 94).

Liposomes are one, if not the most, well-studied class of drug delivery systems. It reached the clinic in the 1990s, with the approval of liposomal doxorubicin. The formulation exhibited increased therapeutic effects and

decreased toxicity compared to free doxorubicin, which included serious side effects such as congestive heart failure (95).

The main drawbacks of liposomes are limited encapsulation of hydrophobic and amphiphilic drugs, and the relatively large size of the structure. The latter tends to hinder deep penetration into tumor tissue. Furthermore, when liposomes have a diameter greater than approximately 100 nm, their ability to diffuse rapidly is reduced and EPR-mediated accumulation in the tumor may be hampered (91).

Lipid bilayer disks (lipodisks)

Lipid bilayer disks (lipodisks) are nanosized bilayer structures, stabilized into flat, circular shapes by PEG-linked lipids (96-98). These structures are generally smaller than the spherical-shaped liposomes (Figure 5).

The two main components of lipodisks are phospholipids and PEG-modified lipids, where the latter are responsible for creating a curvature at, and stabilizing, the rim of the disk. By attaching a targeting moiety to the PEGylated lipids, the lipodisks can be directed toward tumor cells (99-101).

Lipodisks exhibit great potential as drug carriers and have been preclinically assessed for delivery of anti-cancer and anti-bacterial compounds (98-103), focusing on amphiphilic and hydrophobic compounds. The benefits of lipodisks over liposomes include a potentially enhanced EPR effect due to the non-spherical shape and smaller size of the lipodisks, as well as higher maximum drug-to-lipid ratio for drugs bound to, or incorporated in, the lipid bilayer (91).

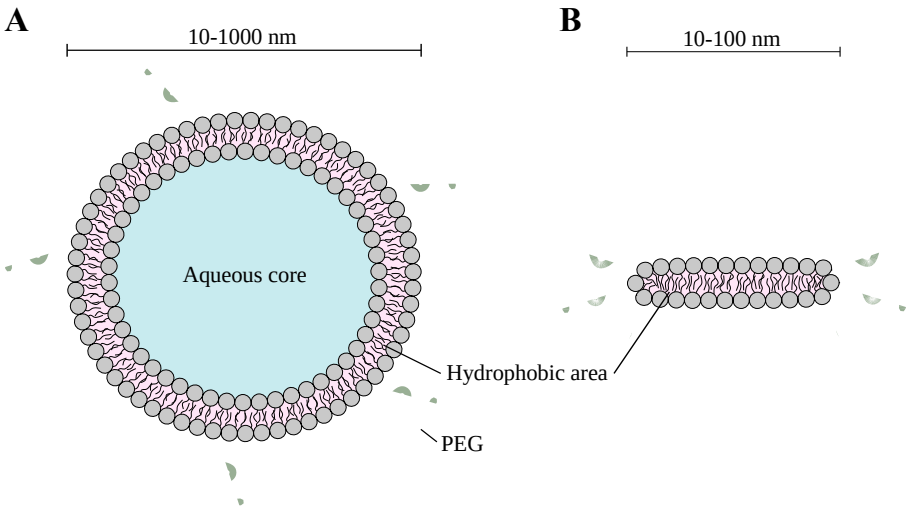

Figure 5: Lipid-based delivery systems. Cross-section of (A) a liposome and (B) a lipodisk. Phospholipids are highlighted in grey, PEG in green, hydrophilic regions in blue and hydrophobic regions in pink.

Radiosensitization strategies

Radiotherapy is a strong weapon against cancer, but it has limitations. Radioresistant subpopulations of tumor cells and normal tissue toxicity limit its efficacy (37). Therefore, it is beneficial to develop tools to overcome these limitations. The use of radiosensitizing drugs is one approach, where pathways involved in the radiation response are altered to enhance the radiation effects (104). The two radiosensitization strategies utilized in this thesis were HSP90 inhibition and p53 stabilization.

HSP90 inhibition

HSP90 inhibition affects several proteins involved in the DDR pathway and can therefore influence the cellular response to radiation (Figure 6) (14). Preclinical research has indeed proved that HSP90 inhibitors act as radiosensitizers for EBRT, via various mechanisms (105-112). For example, HSP90 client protein protein kinase B (AKT) reduce the effects of radiotherapy by increasing DNA-PKcs activity (111, 113).

Furthermore, both MDM2 and p53 are client proteins of HSP90 (114, 115). Interestingly, HSP90 inhibition has been reported to have differing effects on p53, depending on the mutational status, with upregulation of wildtype (wt) p53 and downregulation of the mutated protein (116). The radiosensitizing effects of HSP90 inhibition are indicated to be, at least partly, p53-independent (108, 109).

One study has indicated HSP90 inhibition to be a feasible radiosensitizer together with ^{177}Lu-DOTATATE (117). This, together with the encouraging data from EBRT studies, demonstrates there is great potential for HSP90-mediated radiosensitization in TRT.

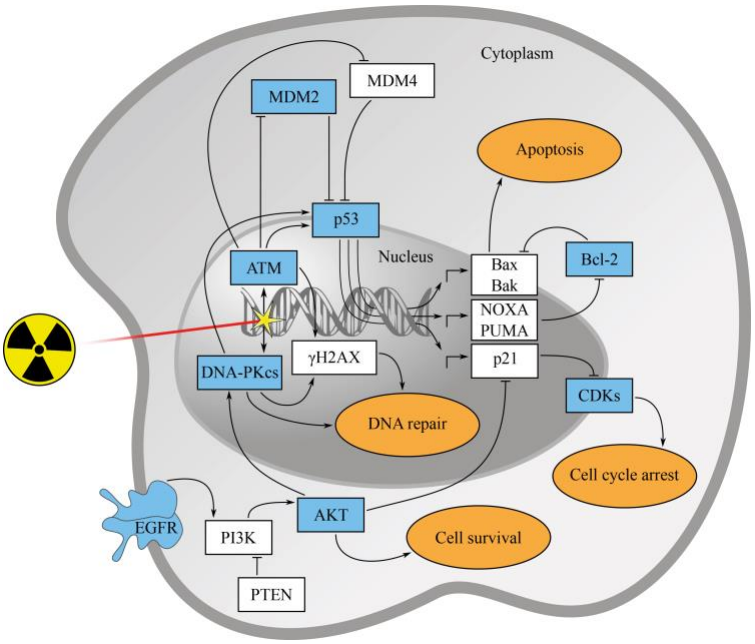

Figure 6: Involvement of HSP90 in DDR. HSP90 client proteins are marked in blue.

p53 stabilization

The involvement of p53 in the response to radiation is extensive and central, as described above. Thus, the use of p53-stabilizing compounds, e.g., MDM2 inhibitors, as radiosensitizers is a rational approach. Several MDM2 inhibitors mediate p53-specific radiosensitization when combined with EBRT (118-122). Furthermore, the stapled peptide PM2, a predecessor or VIP116, exhibits radiosenstizing properties, in combination with both EBRT and TRT (123-125).

p53-mediated radiosensitization has been proposed to act through several pathways, initiated by an increase in the p53 levels. This leads to a plethora of responses including, but not limited to, cell cycle arrest, and upregulation of pro-apoptotic and pro-senescence pathways (121, 122, 124). Furthermore, as the transcription of a large number of proteins are controlled by p53, the radiosensitization mechanisms can rely on additional pathways, e.g., DNA repair kinetics (119).

One study has investigated the effects of the MDM2 inhibitor Nutlin-3 in combination with ^{177}Lu-DOTATATE in a panel of neuroblastoma cell lines, with promising results (54). However, the radiobiology of p53-mediated radiosensitization in TRT remains greatly uncharted.

Investigation of drug synergy

When combining radiotherapy with a potentially radiosensitizing drug, it is relevant to assess whether a combination is more beneficial than the monotherapies. Perhaps perceived as a simple question at a first glance, it has proven hard to translate the theory of drug synergy into practice. The assessment of drug interactions is based on a definition of additivity, the "reference" state where two drugs are neither synergistic nor antagonistic. There is no consensus definition of additivity; as a result, several synergy models have been developed, all based on different mathematical definitions of additivity (126, 127).

Effect based synergy models

Effect-based models compare the effect of a combination therapy to those of the corresponding monotherapies. These types of models include Combination Subthresholding, Highest Single Agent (HSA), Response Additivity, and Bliss Independence. The main advantage of using effect-based models is the low requirement for input data, as synergy can be assessed in situations where only one dose of each drug is assessed (126, 127). The Bliss Independence model, which was applied in paper III, defines additivity as

$$\frac{E_{drug\ A} + E_{drug\ B} - E_{Drug\ A} * E_{drug\ B}}{E_{combination}} = 1 \qquad (1)$$

where E is the effect of the respective treatment. The Bliss model assumes that drugs act independently, which can pose a problem when investigating drugs with unknown mechanisms of action. Furthermore, several effect-based models are unable to take the curvilinear shape of dose-response curves, as is the case for most drugs, into account. This affects the quality of the calculations (126, 127).

Dose-effect based synergy models

Dose-effect-based models rely on the dose-response curves of the monotherapies in addition to effects, which is the sole input in effect-based models (126, 127). Most dose-effect models are based on Loewe Additivity, which is defined as

$$\frac{CD_{drug\ A}}{MD_{drug\ A}} + \frac{CD_{drug\ B}}{MD_{drug\ B}} = 1 \qquad (2)$$

where CD is the dose of each drug, which in combination gives a specific effect. MD is the dose required for each drug as a monotherapy to achieve the same effect. The most well-known synergy model based on Loewe Additivity is the Median Effect/Chou-Talalay model (128, 129). This model was used for synergy calculations in paper I.

The limitations of the dose-effect based models include the necessity for additional input data, as these models require accurate dose-response curves for each compound. Furthermore, most models assume, or perform best with, a constant potency-ratio between drugs, i.e., parallel dose-response curves (126, 127).

As a mean to address the difficulties with differing potency-ratios of dose-response curves, the Zero Interaction Potential (ZIP) model was introduced in 2015 (130). Here, the differences in potency between mono- and combination therapies are taken into account, and the model is proposed to combine the qualities of Bliss- and Loewe-based synergy models. This was, in addition to Bliss Independence, applied in paper III.

Preclinical models for the study of cancer used in the present work

In vitro models

Ever since the establishment of the first human cancer cell line, known as HeLa, in 1951, *in vitro* culture of cancer cells has been essential for drug discovery (131). This technique comes with the advantages of low costs and high through-put (132). There are numerous commercial cell lines available for most, if not all, types of tumors (133).

The approval rate for anticancer drugs after entering phase 1 trials is reported to be only 5–7.5%. This is partly attributed to the use of monolayer tumor cell cultures (132, 134). Therefore, culturing the cancer cells in 3D as multicellular tumor spheroids has emerged. In contrast to monolayer cultures, spheroids have oxygen and nutrient gradients, and can be co-cultured with non-tumor cells in extra-cellular matrix (ECM)-like structures (132). Spheroids consisting of several cell types and grown in a matrix are sometimes referred to as organoids (135). There are numerous techniques to obtain a spheroid, spanning from the use of ultra-low attachment plates to 3D-printing of cells (132, 134, 136).

Spheroids are particularly suitable for the study of radionuclide therapy, as the radiation energy is deposited in all directions from the decay. Moreover, the presence of an oxygen gradient is important, as hypoxic cells are less susceptible to γ and β radiation (137).

Although the use of spheroids is considered as more *in vivo*-like than monolayer cultures, there are limitations with this model as well. Spheroid

cultures generally require more time, work, and resources compared to monolayer cultures. Furthermore, analytical methods such as colorimetric or fluorescent-based measurements may be difficult due to decreased penetration of the reagents into the spheroid (132).

Both monolayer and spheroid cultures are included in the present work. The techniques were applied in a complementary manner, where monolayer cells were used for short-term assays, mainly for characterizations of cell lines. Spheroids were then used for therapeutic experiments, looking at growth-inhibitory effects from treatment over a longer time period. Additionally, a majority of the molecular analysis, e.g., western blot and flow cytometry, were done on cells grown as spheroids.

In vivo models

When the *in vitro* part of a study presents with a promising drug candidate, it can be further evaluated *in vivo*. This can provide information on drug distribution and clearance as well as potential toxicities. In cancer research, mice are the most common animal model, having a high genetic similarity to humans while being cost effective. However, the use of animal models comes with several ethical and biological considerations of their suitability as models for human cancer. Despite these issues, it is considered to be the best approach to evaluate novel drug candidates at the moment. There are several techniques to establish a cancer mouse model, e.g., injection of human (xenograft) or murine (allograft) cancer cells, treatment with carcinogens, and genetic modifications (138, 139).

In the current study, immune-deficient mice with human xenografts were used. This model is established relatively fast, with tumor engraftment within 7–30 days after injection. As the tumors are of human origin, the drug targets are genetically correct. This model, however, does not capture the true course of tumorigenesis, as the xenograft tumors grow much faster than normal. They are also not situated in a clinically relevant location for the studied tumor types, as cells are injected subcutaneously on the hind leg flank. Moreover, immunodeficient mice cannot be used to fully study the immune system's involvement in therapy response.

Aim of the thesis

This thesis aimed to assess potential radiosensitizing strategies for ^{177}Lu-DOTATATE treatment in cancer, focusing on HSP90 inhibition and p53 stabilization.

Results and discussion

Papers I and II: HSP90-mediated radiosensitization of ^{177}Lu-DOTATATE therapy in NETs

^{177}Lu-DOTATATE is approved for therapy of gastroenteropancreatic NETs, with impressive response rates (9, 55). Complete response, however, is seldom achieved (55, 57-59). In papers I and II, we aimed to address this by investigating the HSP90 inhibitor Onalespib, described in more detail above, as a treatment option for NETs and as a potentiator of ^{177}Lu-DOTATATE. HSP90 inhibition has previously been proven to have radiosensitizing properties, and it is a promising therapeutic target for neuroendocrine cancer (10-14).

In paper I, the combination therapy was assessed *in vitro*. We characterized a set of NET cell lines for ^{177}Lu-DOTATATE uptake and sensitivity towards Onalespib in monolayer cultures. The cells were then cultured as multicellular tumor spheroids, and growth inhibitory effects from mono- and combination therapies were assessed.

Onalespib had growth inhibitory effects, leading to a halted spheroid growth in all studied cell lines. ^{177}Lu-DOTATATE was able to reduce spheroid growth in SSTR positive cell lines BON and NCI-H727, while SSTR negative NCI-H460 was unaffected. The combination therapy followed the same pattern (Figure 7). Onalespib potentiated ^{177}Lu-DOTATATE in BON and NCI-H727 (Figure 7A-F), while the effect combination therapy did not differ from those of Onalespib monotherapy in NCI-H460 (Figure 7G-I).

The various combinations tested in the two SSTR positive cell lines were assessed for synergy. Using the Median Effect synergy model, the combination was concluded to be synergistic in BON and NCI-H727 on day 20. On day 14, however, lower doses of Onalespib and ^{177}Lu-DOTATATE were not synergistic in NCI-H727.

Western blot and flow cytometry analyses focused on HSP90 client protein signaling and induction of apoptosis (Figure 8). Onalespib and combination treatment resulted in downregulation of EGFR and subsequent repression of the PI3K/AKT and mTOR pathways. Furthermore, upregulation of Caspase 3/7 implied increased apoptosis in treated spheroids, with the strongest response in the combination group.

The results from paper I support the rationale for utilizing HSP90 inhibition, both as a monotherapy for NETs and as a radiosensitizer of ^{177}Lu-DOTATATE. Our work highlighted some key features that indicate HSP90 inhibition to affect DDR, i.e., through EGFR suppression.

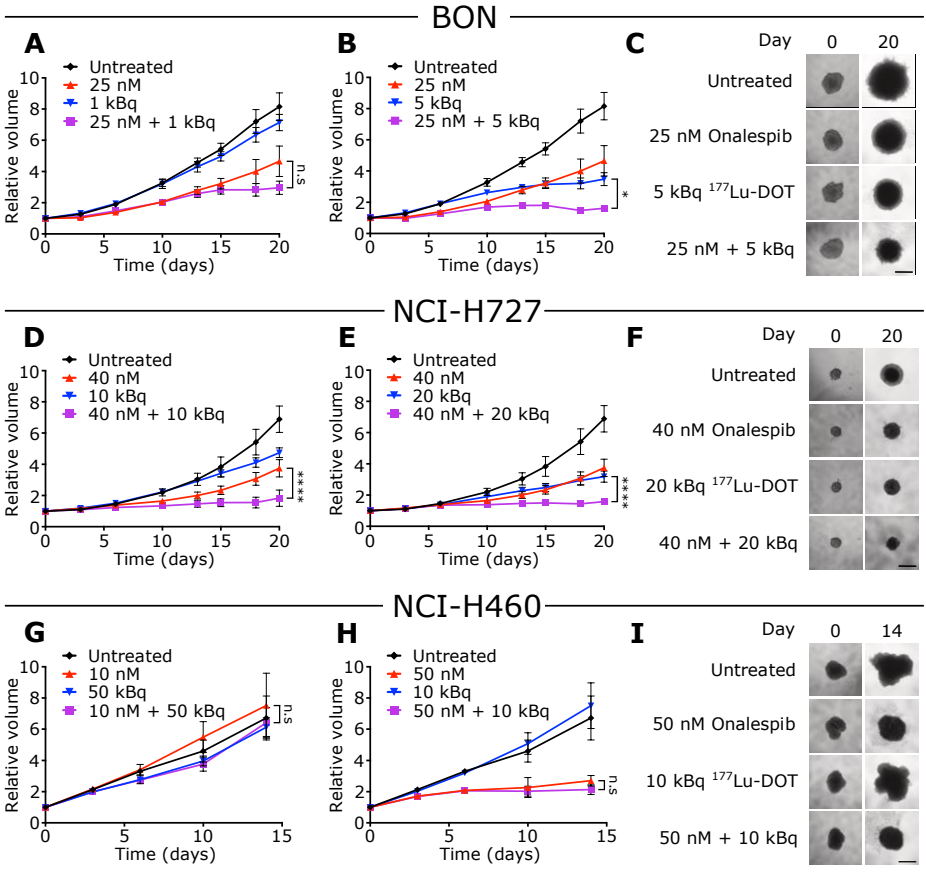

Figure 7: Therapy of Onalespib (▲), ^{177}Lu-DOTATATE (▼) and the combination of the two (■) in SSTR positive (A-C) BON, (D-F) NCI-H727 and SSTR negative (G-I) NCI-H460 spheroids. Graphs display mean ± SD, N ≥ 4. Representative spheroid images are from the first and last time point, scale bar = 500 µm. *P ≤ 0.05, ****P ≤ 0.0001, n.s., not significant.

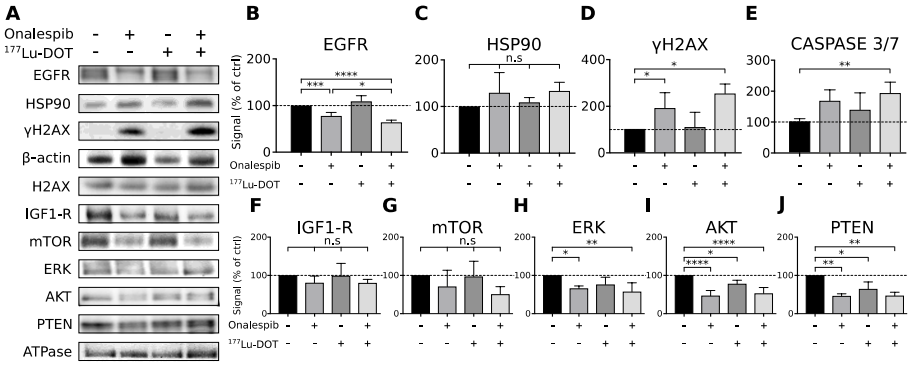

Figure 8: Western blot and flow cytometry analysis 24 hours after final treatment. (A) Representative western blot images of EGFR, HSP90, γH2AX, H2AX, IGFR, mTOR, ERK, AKT, PTEN, and loading controls (β-actin and ATPase). (B-D, F-J) Western blot quantifications, normalized to untreated control (mean ± SD). γH2AX was normalized to unphosphorylated H2AX. (E) Flow cytometry of caspase 3/7. *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001, n.s, not significant.

In paper II, we expanded the investigations with an *in vivo* xenograft mouse model, assessing the efficacy of the treatments as well as molecular analysis of therapeutic response and toxicity. The *in vivo* xenograft study correlated well with results from the *in vitro* assays in paper I. Onalespib and ^{177}Lu-DOTATATE demonstrated growth inhibitory effects as monotherapies, whereas the combination proved to be more potent (Figure 9A). The complete remission rates were 29% in the combination group, compared to 0% and 8% in Onalespib and ^{177}Lu-DOTATATE group, respectively (Figure 9B). The complete remissions were confirmed with SPECT/CT (Figure 10) and dissection.

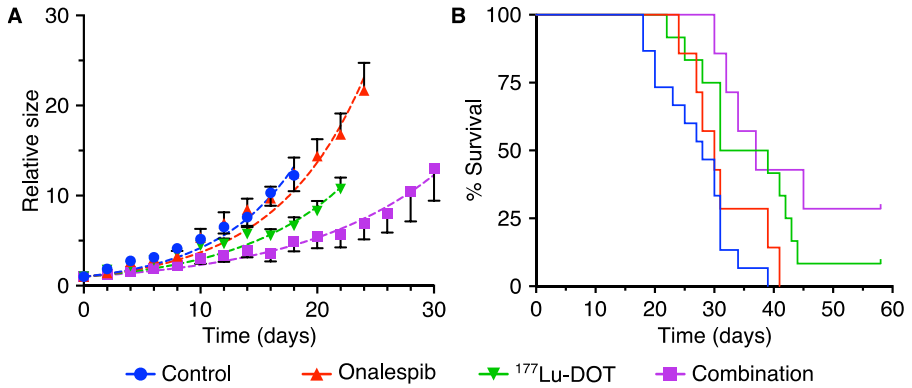

Figure 9: *In vivo* xenograft study. (A) Tumor growth over time (mean + SEM. N ≥ 7). Data were fitted to an exponential growth curve (dashed line). (B) Survival proportions of mice (N ≥ 7).

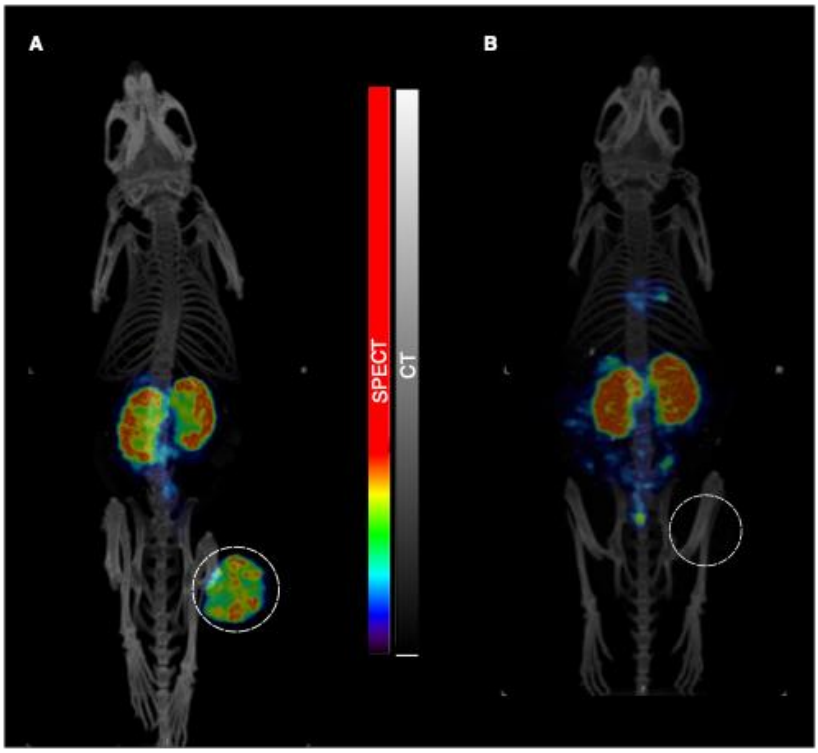

Figure 10: SPECT/CT images at endpoint for (A) mouse sacrificed due to size of tumor and (B) mouse reaching complete remission.

The renal toxicity profile, assessed in paper II, was surprisingly favorable for the combination group (Figure 11). ^{177}Lu-DOTATATE induced damage in the form of glomerular contraction, but this was not present in the combination group (Figure 11A-B). Thus, while having radiosensitizing properties in the tumor, it seemed that Onalespib had a radioprotective effect in the kidneys. We hypothesized that this may be due to induction of heat shock protein 70 (HSP70), which is part of the endogenous stress response to renal injury (140-142). Indeed, the expression of HSP70 was upregulated in the kidney of Onalespib and combination treated mice (Figure 11C-D).

The toxicity profile was encouraging, as one major issue of combination therapies is overlapping side effects. If Onalespib indeed can protect from radiation-induced renal toxicity, it may be possible to increase the dose of ^{177}Lu-DOTATATE without risking additional damage to the kidneys. Further investigations are warranted to characterize the potential radioprotective effect of Onalespib, which should focus on functional studies on the kidneys.

In conclusion, in papers I and II, we were able to demonstrate the feasibility of using Onalespib as a potentiator of ^{177}Lu-DOTATATE therapy, resulting in synergistic effects and with a favorable toxicity profile.

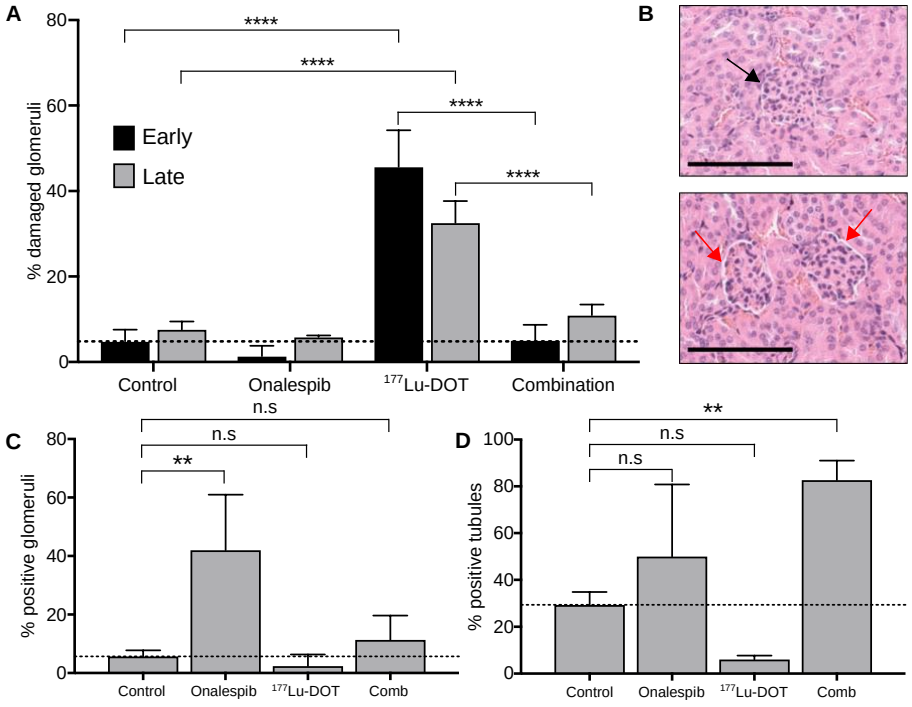

Figure 11: *Ex vivo* immunohistochemical and histological analysis. (A) Quantification of glomerular damage (mean + SD. N = 3). (B) Representative images of normal (top image, black arrow) and damaged (bottom image, red arrows) glomeruli. Bar = 100 µm. (C) Quantification of staining extent of HSP70 positive glomeruli (mean + SD. N = 3). (D) Quantification of staining extent of HSP70 positive tubules (mean, SD. N = 3). n.s = not significant, ** $p < 0.01$, **** $p < 0.0001$.

Paper III: p53-mediated radiosensitization of ^{177}Lu-DOTATATE in neuroblastoma

In paper III, we wanted to potentiate ^{177}Lu-DOTATATE in neuroblastoma, a disease group which frequently presents with SSTR2 upregulation (143-149). As a potential radiosensitizer, we chose the novel MDM2/MDM4 inhibitor VIP116, described in more detail above. Neuroblastomas are rarely mutated in the p53 gene, which makes them suitable for therapy with MDM2 inhibitors (150-157). Furthermore, an earlier version of VIP116 has previously been assessed as a radiosensitizer of EBRT and TRT, with encouraging results (123-125).

A panel of neuroblastoma cell lines was characterized for ^{177}Lu-DOTATATE uptake and sensitivity toward VIP116 in monolayer cultures. SSTR-positive and wtp53 cell lines IMR-32, LU-NB-1 (NB1), and LU-NB-2 (NB2) were cultured as multicellular tumor spheroids and treated with ^{177}Lu-DOTATATE or VIP116. All cell lines responded well to the monotherapies.

Furthermore, IMR-32 and NB2 were treated with a combination modality (Figure 12), demonstrating synergy in IMR-32 and additivity in NB2 spheroids. The synergy was calculated with the Bliss and ZIP synergy models using 25 dose combinations in each cell line.

The method for assessing synergy in this paper was different from paper I. In paper III, an effort was made to study the synergy over a larger dose area. Additionally, the current setup was compatible with the Synergyfinder software, enabling semi-automated synergy calculations for a large number of combinations using several synergy models. As a conclusion, the synergy assessment in this project can be seen as more comprehensive compared to that in paper I.

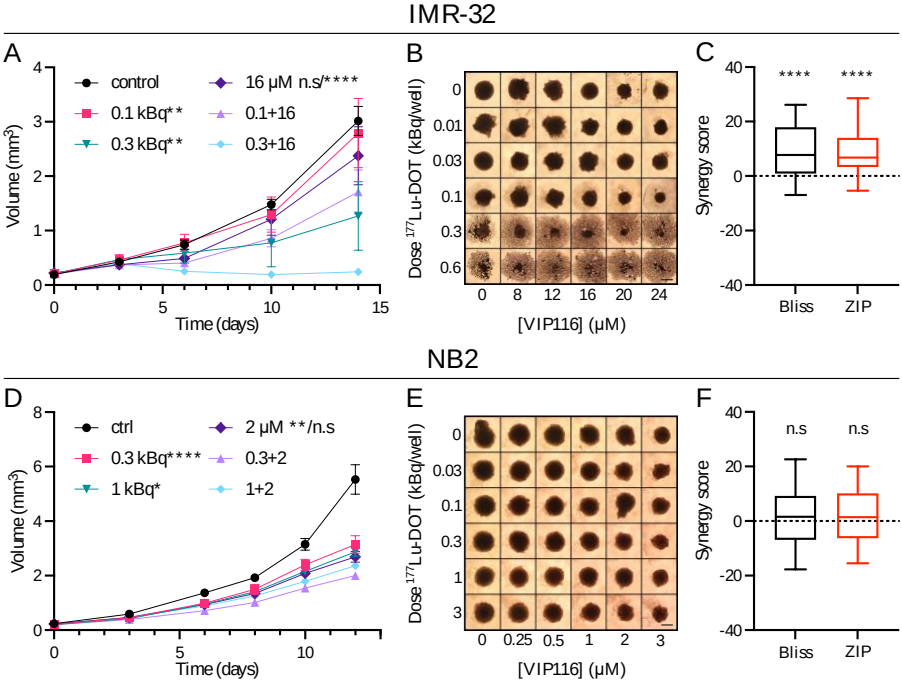

Figure 12: Spheroid combination therapy. (A, D) Representative graphs (mean ± SD, n ≥ 4) of spheroids treated with ^{177}Lu-DOTATATE (■ and ▼, unit kBq), VIP116 (◆, unit µM) and the combination of the two (▲ and ◆). P-value indications next to monotherapy legends are in comparison to the corresponding combination group(s). (B, E) Representative spheroid images at last time point. Scale bar = 500 µm. (C, F) Synergy values for spheroid combinations using Bliss (black) or ZIP (red) synergy models. Values below 0 indicate antagonism, 0 indicates additive effect, and above 0 indicates synergy. Pooled data from two independent experiments (n = 50) are presented as mean with box (25th to 75th percentile) and whiskers (min to max). n.s. = not significant, * = $p < 0.05$, ** = $p < 0.01$ and **** = $p < 0.0001$.

To investigate the radiosensitization mechanism, molecular analysis of the p53 pathway was performed (Figure 13). VIP116 and combination therapy induced p53 with downstream activation of cell cycle (p21) and apoptosis (cleaved caspase 3 and Bax) regulators. Moreover, the activation of p53-repressor MDM2 differed greatly between IMR-32 and NB2. We speculated that this may explain the varying degree of synergy and might be a result of differential MYCN expression.

The results from this study validated the use of p53-stabilizing compounds as radiosensitizers of TRT in neuroblastoma. By adding VIP116, the ^{177}Lu-DOTATATE activity could be reduced by a third, without compromising the therapeutic effect. If this can be translated into a clinical setting, it could reduce the adverse effects of radiotherapy, which is especially important in pediatric patients. Furthermore, kinetics of p53 induction were assessed and revealed a possible involvement of MYCN (summarized in Figure 14). As MYCN is highly involved in the biology of neuroblastoma, this should be further investigated.

In conclusion, VIP116 potentiated ^{177}Lu-DOTATATE therapy in neuroblastoma spheroids and may be a feasible treatment option for this tumor type. Additional *in vivo* assessments are warranted to verify these promising results as well as to assess potential toxicity. Our findings also highlighted the importance of further exploring the p53-MDM2-MYCN interplay in response to radiation in neuroblastoma.

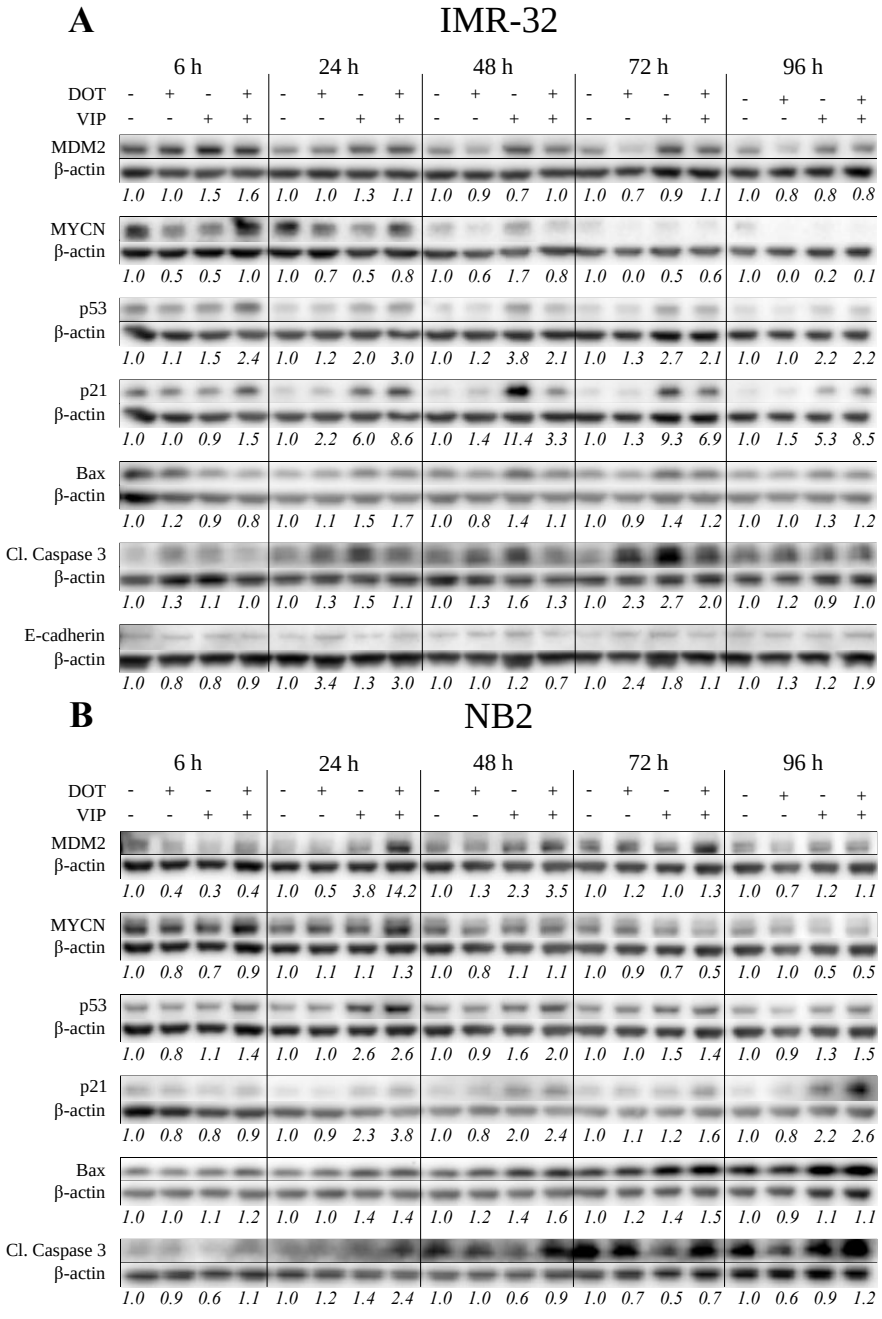

Figure 13: Western blot analysis of p53 activation. Expression of MDM2, MYCN, p53, p21, Bax, cleaved caspase 3, and E-cadherin in (A) IMR-32 and (B) NB2 6–96 h after treatment with ^{177}Lu-DOTATATE and/or VIP116. Representative data from one experiment. Intensity values, shown below each band, were normalized against the loading control (β-actin) and the corresponding untreated control for each time point.

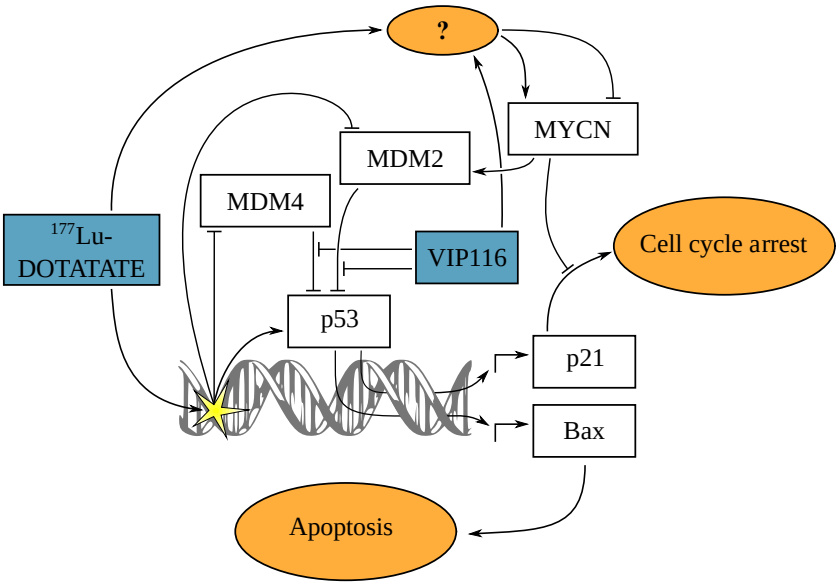

Figure 14: Suggested mechanism of VIP116-mediated potentiation of ^{177}Lu-DOTATATE in neuroblastoma. ^{177}Lu-DOTATATE causes DSBs which initiate a slight p53-mediated response. VIP116 is able to enhance the p53 response and induce transcription of downstream targets, leading to increased apoptosis. MYCN, which can be induced or repressed by the therapies via unknown mechanisms, can affect p53 function negatively via MDM2 transcription or blocking p21-mediated cell cycle arrest.

Paper IV: Targeted delivery of p53-stabilizing peptide VIP116 with lipodisks

In paper IV, we addressed the limited water solubility and lack of tumor-targeted delivery of VIP116. As this may affect its *in vivo* efficacy, we aimed to improve these issues using lipid bilayer disks (lipodisks) as a drug delivery system. We attached the epidermal growth factor (EGF) to the lipodisks, for specific delivery. EGF binds to EGFR, a receptor commonly overexpressed in cancer (158). Lipodisks have previously been assessed in a preclinical setting for delivery of anti-cancer and anti-bacterial compounds (98-103).

Lipodisks were produced using detergent depletion. The VIP116-loading capacity of the lipodisks was characterized by quartz crystal microbalance with dissipation monitoring (QCM-D, Figure 15A-B), resulting in a maximum peptide-to-lipid ratio (R_{eff}) of 0.11. The binding between the lipodisks and VIP116 did not follow a 1:1 interaction model; therefore, we sought to assess the affinity toward the different parts of the lipodisk (Figure 15C). We

concluded that there were only minor interactions between the peptides and the flat part of the lipodisk, as represented by a lipid bilayer membrane.

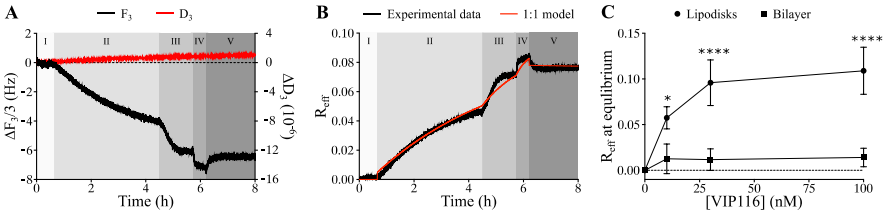

Figure 15: Interaction between lipodisks of VIP116. (A) Raw QCM-D data displaying the normalized frequency (ΔF/3, black) and dissipation factor (ΔD, red) measured at the 3rd overtone. (B) R_{eff} as a function of time calculated from the QCM-D data (black) and fitted to a 1:1 binding model (red). (C) Binding isotherm of VIP116 to lipodisks (circles) and to a supported lipid bilayer (squares), (mean ± SD. N = 3). Solid lines are a guide to the eye. * $p < 0.05$, **** $p < 0.0001$.

This indicated that the rim of the lipodisk was the major binding site for VIP116. Binding may include both binding to the PEG-rich rim and subsequent displacement of the peptide into the lipodisk core, which would explain the lack of 1:1 binding. If VIP116 is incorporated in the lipodisk core, using larger lipodisks may increase the R_{eff}.

The *in vitro* cellular binding of ^{125}I-labeled VIP116 loaded onto EGFR-targeting lipodisks, measured with LigandTracer, was significantly higher than that observed when using untargeted lipodisks, proving the specificity of the system (Figure 16A). The uptake correlated well with the binding pattern of EGF (Figure 16B). Moreover, VIP116-loaded EGFR-targeting lipodisks were able to reduce tumor cell viability, while VIP116-loaded untargeted lipodisks did not have an effect (Figure 16C).

The results obtained in paper IV provide a proof-of-concept that lipodisks can be used for targeted delivery of VIP116. However, before the system can be expanded to *in vivo* model systems and clinical use, several issues have to be addressed. First, the production method for EGF-conjugated lipodisks used here was laborious with low EGF-conjugation yields, making upscaling difficult and expensive. Second, the EGF-EGFR targeting system may not be optimal for delivery of p53-stabilizing compounds, as there are very few cancer cell lines with overexpression of EGFR and wtp53. Thus, we created an EGFR-overexpressing wtp53 cell line for this study. The lack of co-existing EGFR amplification with wtp53 in cell lines is supported by data from the cBioPortal database, where only 3% of the 25,965 available samples have a high-level amplification of EGFR, together with wtp53 (159, 160). This rate is albeit higher for specific types of tumors, such as glioblastoma. Third, it has been reported that PEG may have immunogenic properties, which can hinder its clinical suitability (161). However, this may not be an issue for smaller

PEG-ylated structures like lipodisks, as the PEG-induced immune response appears to increase with nanoparticle size (162).

In conclusion, we proved the feasibility of using targeted lipodisks as a delivery system of VIP116. In the future, this system can be expanded to include additional drugs and targeting moieties to increase efficacy and areas of use.

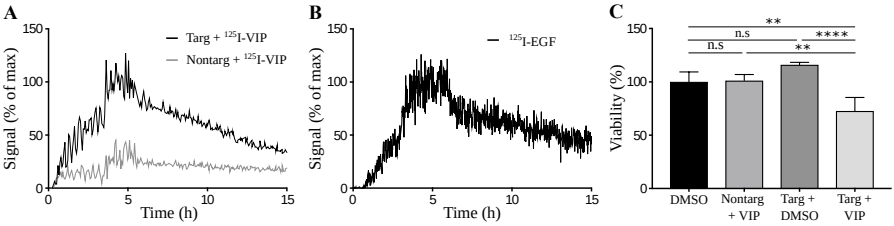

Figure 16: Real-time binding and viability assays of VIP116-loaded lipodisks on EGFR-expressing HCT116 cells. (A) Real-time binding of ^{125}I-VIP116 loaded on EGFR-targeted (black) and non-targeting (grey) lipodisks. Binding was studied for three consecutive concentrations, followed by a dissociation phase. (B) Real-time binding of ^{125}I-EGF. (C) XTT viability assays, demonstrating reduced cell viability in cells treated with EGFR-targeted lipodisks carrying VIP116, whereas no effects were observed from either non-targeted lipodisks carrying VIP116, or from EGFR-targeted lipodisks carrying no VIP116 (mean ± SD. N = 3). Nontarg = non-targeting lipodisks. Targ = targeting lipodisks. VIP =VIP116. ** $p < 0.01$, **** $p < 0.0001$.

Conclusion

This thesis has addressed potential radiosensitizing strategies, i.e., HSP90 inhibition and p53 stabilization, for ^{177}Lu-DOTATATE treatment in NETs and neuroblastoma, respectively.

In papers I and II, we concluded that the HSP90 inhibitor Onalespib can act as a radiosensitizer in NETs when combined with ^{177}Lu-DOTATATE. The combination was synergistic, with a favorable toxicity profile. Inhibition of EGFR was suggested as a mediator of the observed synergy.

In paper III, we used the p53-stabilizing stapled peptide VIP116 to potentiate ^{177}Lu-DOTATATE in neuroblastoma. Combination therapy exhibited promising effects, with resulting additive or synergistic effects. The treatment-mediated effects on p53 signaling were characterized, revealing a possible involvement of MYCN.

In paper IV, we aimed to improve delivery of VIP116. VIP116 was successfully loaded onto EGFR-targeting lipodisks, leading to specific delivery and reduction of viability of EGFR positive tumor cells. The study provided proof-of-concept for utilizing lipodisks as a drug delivery system for p53-stabilizing peptides.

In conclusion, we have investigated, and found, suitable candidates for potentiating ^{177}Lu-DOTATATE therapy. We have addressed several important questions regarding the feasibility of the treatments, toxicity, and targeted delivery. Moreover, the work has explored the biology of radionuclide therapy. This is an area in need of further attention, as more and more radionuclide therapies are currently entering clinicals trials and reaching approval.

Future perspectives

The field of TRT has received increased attention over the last 10 years, with several TRT compounds reaching clinical approval. In the near future, the list of approved TRTs is expected to be expanded with new compounds, e.g., ^{177}Lu-PSMA-617 (22). With this renewed attention, there is a need to understand the radiobiology of the therapies. Additionally, the use of combination strategies should be further investigated, in order to optimize the therapeutic effect and reduce toxicity.

The use of combination approaches for TRT is currently being explored in several areas. This thesis focused on radiosensitization by targeting two proteins affecting the DDR. However, there are several additional approaches including but not limited to, improved distribution and uptake of the TRT compound, and combinations with other DNA-damaging agents or immunotherapy (163). It is known that radiation may cause a release of immune cell-attracting cytokines which can lead to immunogenic cell death, making it a highly relevant target for combination therapy with TRT (164).

This thesis did not address the immunological response to radiation, mainly because the *in vivo* studies were conducted in immune-deficient mice. Nevertheless, the work is a basis for further investigations in immune-competent models, which are currently being conducted by our research group. There are several ongoing projects, including potentiation of ^{177}Lu-DOTATATE in neuroblastoma using immunotherapy. Furthermore, as both HSP90 inhibition and p53 stabilization enhances cancer immunotherapy, there is also a possibility to explore a triple combination of radiation, a radiosensitizer, and immune therapy (165, 166).

In addition to combination therapy, the use of α-emitting radionuclides is currently on the rise. With their short range and capability to induce complex, irreparable DSBs, they have exhibited efficacy against widespread disease (167). ^{213}Bi- and ^{225}Ac-DOTATATE have been assessed in a clinical setting, with positive results (62, 168-170).

The preclinical results presented here have great potential to be translated into clinical use. ^{177}Lu-DOTATATE is already approved for therapy of gastroenteropancreatic neuroendocrine cancer, where it has displayed anti-tumor effects with a manageable toxicity profile. It is currently being explored for additional tumor types, including the disease of focus in paper III, neuroblastoma (144, 147, 148).

Moreover, the use of p53-stabilizing agents for treatment of neuroblastoma also displays great potential. The stapled peptide ALRN-6924, the same type of molecule as our p53-stabilizing peptide VIP116, is currently undergoing phase 1 studies in pediatric tumors with wtp53 (70).

A total of 18 HSP90 inhibitors have entered clinical trials (69, 70), resulting in a broad knowledge base regarding the benefits and challenges of targeting HSP90. There are several novel compounds currently in phase 1 studies, which hopefully are able to circumvent the adverse effects accompanying HSP90 inhibition. Moreover, Onalespib is currently being assessed in combination with radiotherapy and cisplatin for treatment of head and neck carcinoma in a phase 1 clinical study.

In the light of these ongoing clinical studies, there is a clear potential for the radiosensitizers studied in this thesis. Nevertheless, issues concerning toxicity and dosing scheduling must be further addressed before the combination therapies with ^{177}Lu-DOTATATE can be used in a broad clinical setting. In the future, we hope that our efforts can improve the therapeutic outcome, and life, for cancer patients who currently do not have sufficient treatment options available.

Populärvetenskaplig sammanfattning

Cancer är en sjukdom som orsakar mer än nio miljoner dödsfall varje år i världen. I Sverige får en av tre ett cancerbesked under sitt liv. Alla former av cancer har en gemensam nämnare, obehindrad celldelning, men kan ha väldigt olika egenskaper. Cancersjukdomar grupperas därför efter var de uppstår och vilka typer av celler de härstammar från.

De vanligaste behandlingarna mot cancer är kirurgi, cellgifter och strålbehandling. Ungefär hälften av alla patienter med cancer får strålbehandling någon gång under behandlingsprocessen. Det kan antingen ges externt, genom att en strålkälla utanför kroppen riktas mot tumörer, eller internt, då strålkällan placeras inuti kroppen. Den primära effekten av strålbehandling är DNA-skador, vilket kan orsaka celldöd. Både tumörceller och friska celler påverkas av strålning, men tumörceller är mer känsliga eftersom de inte har samma kapacitet att reparera DNA-skador.

Konceptet precisionsmedicin har dominerat forskningen kring nya cancerläkemedel under de senaste decennierna, där fokus ligger på att individanpassa behandling utifrån varje tumörs egenskaper. Det kan till exempel vara användning läkemedel som binder till receptorer, proteiner som finns på tumörcellens yta, och enbart ge läkemedlet till patienter med tumörer som bekräftats ha dessa receptorer.

En form av precisionsmedicin är målsökande radionuklidterapi, vilket är en intern strålbehandling. Läkemedlet består av en cancermålsökande molekyl bunden till en radioaktiv atom. Genom att målsöka en cancerspecifik struktur, till exempel en receptor, ackumuleras strålningen i tumörer medan friska celler skonas. Molekylerna kan inför behandling användas för bilddiagnostik, med en PET eller SPECT-kamera, för att se om patientens tumörer producerar den målsökta strukturen och därmed kan dra nytta av behandlingen.

Den här avhandlingen fokuserar på ^{177}Lu-DOTATATE, en typ av målsökande radionuklidterapi. Den används i cancerbehandling idag, men resulterar sällan i att patienten botas. Målet med avhandlingen var därför att förbättra effekten av ^{177}Lu-DOTATATE genom att kombinera den med ett strålsensitiverande läkemedel, vilket kan påverka tumörcellernas förmåga att skydda sig mot strålbehandling.

I delarbete I och II använde vi ett läkemedel som hämmar heat shock protein 90 (HSP90). HSP90 är ett protein involverat i DNA reparation. Genom att blockera HSP90 kunde vi förstärka effekterna av ^{177}Lu-DOTATATE i

neuroendokrin cancer, en cancertyp som idag behandlas med ^{177}Lu-DOTATATE. Behandlingen resulterade i att flera proteiner, involverade i celldelning och reparation av DNA-skador, som samarbetar med HSP90 bröts ned och tumörerna växte långsammare. Dessutom hade möss som behandlats med kombinationsbehandlingen färre biverkningar än de som behandlats med enbart ^{177}Lu-DOTATATE.

I delarbete III studerade vi strålsensitivering av ^{177}Lu-DOTATATE i högrisk neuroblastom, en allvarlig form av barncancer. Som strålsensitiverare använde vi VIP116, en molekyl som skyddar proteinet p53 från nedbrytning. p53 har en central roll i cellens respons mot DNA skador och kallas därför ”genomets väktare”. Vi fann att kombination av VIP116 och ^{177}Lu-DOTATATE i många fall hindrade tumörcelltillväxt mer än läkemedlen var för sig. Behandlingarna påverkade dessutom MYCN, en markör för dålig prognos i neuroblastom.

VIP116, den p53-stabiliserande molekyl vi undersökte i delarbete III, är inte tumör-specifik. Därför utvärderade vi delarbete IV ett lipid-baserat läkemedelsleverans-system kallat lipodiskar, för att möjliggöra tumör-specifik leverans av VIP116. Vi kunde ladda VIP116 på lipodiskarna och rikta dem mot tumör-specifika receptorer, vilket resulterade i celldöd i tumörceller som producerade receptorn, medan celler utan receptorn skonades.

Sammanfattningsvis har vi i denna avhandling studerat effekter och biverkningar av potentiella radiosensitiverande läkemedel samt möjliggjort specifik leverans av dessa. Vår förhoppning är att dessa resultat i framtiden kan bidra till att människor med neuroendokrin cancer och neuroblastom, som behandlas med ^{177}Lu-DOTATATE, ska svara bättre på behandlingen och slippa allvarliga biverkningar.

Acknowledgements

This thesis was not a one-woman job, it was made possible with the support of many important persons.

First of all, I would like to thank my main supervisor *Marika*, for being a fantastic mentor, letting me do (almost) whatever I want and making my results sound smart. The name of our group, The Best Research Group Ever (TBRGE), demonstrates your leadership skills. I look forward to hearing Marika&The Dorks on my dissertation party.

To my assistant supervisors *Hanna*, for listening to my rants about work and non-work-related issues (although everything is work-related between 8am and 5pm), and *Katarina*, for welcoming me into your world with open arms. The time I spent at BMC has without the doubt been the most rewarding part of my PhD.

To *Anja*, my first supervisor at BMS. You are a fantastic friend and support, despite the facts that you never sleep and always have 400 mice to dissect. To *Diana*, for being the calmest person in the office. Without your sure-we-can-pull-it-off attitude I am not sure this thesis would exist. To *Andris*, for always disagreeing and helping with my Scottish accent. Apologies for totally ripping off your thesis cover.

To the ladies who made TBGRE an actual group rather than a few people in a small conference room. To *Hanna*, the GIF-master. To *Tabassom*, the birthday-surprise general. To *Preeti*, our very own drug designer extra-ordinaire who always brings a smile to work.

To *Sina* and *João*, probably the two smartest people I have ever met. You bring the math to my work, which of course is one of my favorite things next to chocolate. And thank you to the rest of the *Ridgeview team*, for an endless resource of knowledge, tea and great discussions. I look forward to joining you when my PhD comes to an end.

To *Tina*, my work-sister, for letting me come up to the sunny parts of Rudbeck and drink tea even though you never have time. One day, I will pay half your salary and force you to have fika with me two times per day.

To *Bosse*, for having many entertaining citrus-accompanied discussions. To *Vladimir*, our inspiring, Russian bear. To *Kicki, Hadis, Mohammed, Javad, Maryam, Anzhelika, Mehran, Tianqi, Yongsheng, Olga* and *Torbjörn* for making BMS a corridor filled with great science and a positive atmosphere.

To *Victor, Lars, Emma, Sara, Amelie,* and the rest of Edwards group at the chemistry department, for welcoming me to your lab and helping me when I needed it (which was quite often).

To *Svea, Ida, Marcus, Julia,* and the rest of the *IGP PhD council,* for making the last Tuesday of the month a true joy. Without you, I would not have a clue what the rest of IGP does.

To *Claes,* the *IGP administration,* and the *FUG group,* who have taught me more on the topic of PhD studies than my actual PhD studies.

To the people at *PPP,* a place where I have spent more evenings and weekends than I want to admit. Luckily, there is usually someone equally miserable there, so I have rarely been alone!

To *Tobias, Ximena, Maria,* and everyone else at *Molecular Geriatrics,* the kindest (and richest) corridor buddies of all time.

To *Martina 1.0,* my very first office buddy, who I miss every day. Your note is starting to fade, so it is about time you come back and write new ones.

To *Sir David Lane,* TBRGEs very own celebrity, for being such a positive and encouraging collaborator and friend.

To my students, *Anna, Kristina, and Bono,* who did much of the hard work that is the basis of this thesis. I have been lucky to have such fantastic, inspiring students. To *Nakul* (although you were technically not my student), who made TBRGE slightly more masculine and taught a poor engineer how a liver works.

To *Anna Karin* and *Lars* in Umeå, who during the summer of 2012 introduced me to the research, and are the reason I became a PhD student.

To *Lovisa,* who has been taking care of me since we moved to Uppsala (and let's face it, before as well). Without you I wouldn't have passed the first math exam. To her husband *David,* the embodiment of a true researcher. No sense of time management, disordered hair and a curiosity for just about anything. May we one day have the energy and time to go on our second-hand tour in Hälsingland.

To *Josefin, Sofie, Ida, Linn, Maja, Saga, Dennis, Annika, Alice, Kristin,* and all of my friends that I have forgotten to mention here. You make my life fantastic!

Saving the best for last, I would like to thank my family. To my boys, *Johan* and *Ebbe,* for making home the best place on earth. To *Mami,* who forecasted this back in 2007 when I wanted to be a hair-dresser. To *Papa-Bear,* who made sure I had fantastic music in my ears growing up. To my brothers, *Andreas* and *David,* for stop pushing me down the stairs (which may have had a detrimental effect on my PhD-ing capabilities). To my sister-in-laws, *Frida* and *Maja,* who inform me about how proud my brothers are of me even though they never say it directly to me.

And last but not least, thanks to *Rut* and *Knut.* You are featured in one of my illustrations, but since you are cats and will never read this, I don't have to tell you which one.

References

1. Wild CP, Weiderpass E, Stewart BW. World Cancer Report 2020. World Health Organization; 2020.
2. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646-74.
3. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100(1):57-70.
4. Friedman AA, Letai A, Fisher DE, Flaherty KT. Precision medicine for cancer with next-generation functional diagnostics. Nat Rev Cancer. 2015;15(12):747-56.
5. Shin SH, Bode AM, Dong Z. Precision medicine: the foundation of future cancer therapeutics. NPJ Precis Oncol. 2017;1(1):12.
6. Cives M, Strosberg JR. Gastroenteropancreatic Neuroendocrine Tumors. CA: a cancer journal for clinicians. 2018;68(6):471-87.
7. Oronsky B, Ma PC, Morgensztern D, Carter CA. Nothing But NET: A Review of Neuroendocrine Tumors and Carcinomas. Neoplasia. 2017;19(12):991-1002.
8. Frilling A, Akerstrom G, Falconi M, Pavel M, Ramos J, Kidd M, et al. Neuroendocrine tumor disease: an evolving landscape. Endocr Relat Cancer. 2012;19(5):R163-85.
9. Drugs.com. FDA Approves Lutathera (lutetium Lu 177 dotatate) for Gastroenteropancreatic Neuroendocrine Tumors 2018 [Available from: https://www.drugs.com/newdrugs/fda-approves-lutathera-lutetium-lu-177-dotatate-gastroenteropancreatic-neuroendocrine-tumors-4686.html.
10. Gilbert JA, Adhikari LJ, Lloyd RV, Halfdanarson TR, Muders MH, Ames MM. Molecular markers for novel therapeutic strategies in pancreatic endocrine tumors. Pancreas. 2013;42(3):411-21.
11. Gilbert JA, Adhikari LJ, Lloyd RV, Rubin J, Haluska P, Carboni JM, et al. Molecular markers for novel therapies in neuroendocrine (carcinoid) tumors. Endocr Relat Cancer. 2010;17(3):623-36.
12. Gloesenkamp C, Nitzsche B, Lim AR, Normant E, Vosburgh E, Schrader M, et al. Heat shock protein 90 is a promising target for effective growth inhibition of gastrointestinal neuroendocrine tumors. International Journal of Oncology. 2012;40(5):1659-67.
13. Zitzmann K, Ailer G, Vlotides G, Spoettl G, Maurer J, Göke B, et al. Potent antitumor activity of the novel HSP90 inhibitors AUY922 and HSP990 in neuroendocrine carcinoid cells. International Journal of Oncology. 2013;43(6):1824-32.
14. Pennisi R, Ascenzi P, di Masi A. Hsp90: A New Player in DNA Repair? Biomolecules. 2015;5(4):2589-618.
15. Brodeur GM. Neuroblastoma: biological insights into a clinical enigma. Nat Rev Cancer. 2003;3(3):203-16.
16. Pastor ER, Mousa SA. Current management of neuroblastoma and future direction. Crit Rev Oncol Hematol. 2019;138:38-43.

17. Swift CC, Eklund MJ, Kraveka JM, Alazraki AL. Updates in Diagnosis, Management, and Treatment of Neuroblastoma. Radiographics. 2018;38(2):566-80.
18. Zafar A, Wang W, Liu G, Xian W, McKeon F, Zhou J, et al. Targeting the p53-MDM2 pathway for neuroblastoma therapy: Rays of hope. Cancer Lett. 2021;496:16-29.
19. Bailey DL, Humm JL, Todd-Pokropek A, Van Aswegen A, International Atomic Energy A, American Association of Physicists in M. Nuclear medicine physics: a handbook for teachers and students. Vienna: International Atomic Energy Agency; 2014.
20. Chu SYF, Ekström LP, Firestone RB. The Lund/LBNL Nuclear Data Search 1999 [Available from: http://nucleardata.nuclear.lu.se/toi/.
21. Citrin DE. Recent Developments in Radiotherapy. The New England journal of medicine. 2017;377(22):2200-1.
22. Sgouros G, Bodei L, McDevitt MR, Nedrow JR. Radiopharmaceutical therapy in cancer: clinical advances and challenges. Nat Rev Drug Discov. 2020;19(9):589-608.
23. Vallabhajosula S. Molecular Imaging in Oncology. Molecular imaging: Springer; 2009. p. 215-54.
24. Dunne-Daly CF. Principles of radiotherapy and radiobiology. Semin Oncol Nurs. 1999;15(4):250-9.
25. Schipler A, Iliakis G. DNA double-strand-break complexity levels and their possible contributions to the probability for error-prone processing and repair pathway choice. Nucleic Acids Res. 2013;41(16):7589-605.
26. Nickoloff JA, Boss MK, Allen CP, LaRue SM. Translational research in radiation-induced DNA damage signaling and repair. Transl Cancer Res. 2017;6(Suppl 5):S875-S91.
27. Bakkenist CJ, Kastan MB. DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. Nature. 2003;421(6922):499-506.
28. Blackford AN, Jackson SP. ATM, ATR, and DNA-PK: The Trinity at the Heart of the DNA Damage Response. Mol Cell. 2017;66(6):801-17.
29. Merkel O, Taylor N, Prutsch N, Staber PB, Moriggl R, Turner SD, et al. When the guardian sleeps: Reactivation of the p53 pathway in cancer. Mutat Res. 2017;773:1-13.
30. Brown CJ, Lain S, Verma CS, Fersht AR, Lane DP. Awakening guardian angels: drugging the p53 pathway. Nat Rev Cancer. 2009;9(12):862-73.
31. Michael D, Oren M. The p53–Mdm2 module and the ubiquitin system. Seminars in Cancer Biology. 2003;13(1):49-58.
32. Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. Nature. 2000;408(6810):307-10.
33. Wade M, Li YC, Wahl GM. MDM2, MDMX and p53 in oncogenesis and cancer therapy. Nat Rev Cancer. 2013;13(2):83-96.
34. Khoo KH, Verma CS, Lane DP. Drugging the p53 pathway: understanding the route to clinical efficacy. Nature Reviews Drug Discovery. 2014;13(4):314-.
35. Karimian A, Ahmadi Y, Yousefi B. Multiple functions of p21 in cell cycle, apoptosis and transcriptional regulation after DNA damage. DNA Repair (Amst). 2016;42:63-71.
36. Shen Y, White E. p53-dependent apoptosis pathways. Adv Cancer Res. 2001;82:55-84.
37. Gudkov AV, Komarova EA. The role of p53 in determining sensitivity to radiotherapy. Nat Rev Cancer. 2003;3(2):117-29.

38. Ke X, Shen L. Molecular targeted therapy of cancer: The progress and future prospect. Front Lab Med. 2017:69-75.
39. Borras JM, Lievens Y, Dunscombe P, Coffey M, Malicki J, Corral J, et al. The optimal utilization proportion of external beam radiotherapy in European countries: An ESTRO-HERO analysis. Radiother Oncol. 2015;116(1):38-44.
40. Baumann M, Krause M, Overgaard J, Debus J, Bentzen SM, Daartz J, et al. Radiation oncology in the era of precision medicine. Nat Rev Cancer. 2016;16(4):234-49.
41. Tharmalingham H, Hoskin P. The changing role of radiation therapy in the management of oligometastatic disease. Technical Innovations & Patient Support in Radiation Oncology. 2017;1(1):13-5.
42. Zukotynski K, Jadvar H, Capala J, Fahey F. Targeted Radionuclide Therapy: Practical Applications and Future Prospects. Biomark Cancer. 2016;8(Suppl 2):35-8.
43. Langbein T, Weber WA, Eiber M. Future of Theranostics: An Outlook on Precision Oncology in Nuclear Medicine. Journal of nuclear medicine : official publication, Society of Nuclear Medicine. 2019;60(Suppl 2):13S-9S.
44. Rodrigues M, Winkler KK, Svirydenka H, Nilica B, Uprimny C, Virgolini I. Long-Term Survival and Value of (18)F-FDG PET/CT in Patients with Gastroenteropancreatic Neuroendocrine Tumors Treated with Second Peptide Receptor Radionuclide Therapy Course with (177)Lu-DOTATATE. Life (Basel). 2021;11(3).
45. Theodoropoulou M, Stalla GK. Somatostatin receptors: from signaling to clinical practice. Front Neuroendocrinol. 2013;34(3):228-52.
46. Hofland LJ, Lamberts SW. The pathophysiological consequences of somatostatin receptor internalization and resistance. Endocr Rev. 2003;24(1):28-47.
47. Krenning EP, Kooij PP, Bakker WH, Breeman WA, Postema PT, Kwekkeboom DJ, et al. Radiotherapy with a radiolabeled somatostatin analogue, [111In-DTPA-D-Phe1]-octreotide. A case history. Ann N Y Acad Sci. 1994;733:496-506.
48. Orlando C, Raggi CC, Bianchi S, Distante V, Simi L, Vezzosi V, et al. Measurement of somatostatin receptor subtype 2 mRNA in breast cancer and corresponding normal tissue. Endocr Relat Cancer. 2004;11(2):323-32.
49. Chereau E, Durand L, Frati A, Prignon A, Talbot JN, Rouzier R. Correlation of immunohistopathological expression of somatostatin receptor-2 in breast cancer and tumor detection with 68Ga-DOTATOC and 18F-FDG PET imaging in an animal model. Anticancer research. 2013;33(8):3015-9.
50. Dalm SU, Schrijver WA, Sieuwerts AM, Look MP, Ziel-van der Made AC, de Weerd V, et al. Prospects of Targeting the Gastrin Releasing Peptide Receptor and Somatostatin Receptor 2 for Nuclear Imaging and Therapy in Metastatic Breast Cancer. PloS one. 2017;12(1):e0170536.
51. O'Dorisio MS, Chen F, O'Dorisio TM, Wray D, Qualman SJ. Characterization of somatostatin receptors on human neuroblastoma tumors. Cell growth & differentiation : the molecular biology journal of the American Association for Cancer Research. 1994;5(1):1-8.
52. Albers AR, O'Dorisio MS, Balster DA, Caprara M, Gosh P, Chen F, et al. Somatostatin receptor gene expression in neuroblastoma. Regulatory peptides. 2000;88(1-3):61-73.

53. Orlando C, Raggi CC, Bagnoni L, Sestini R, Briganti V, La Cava G, et al. Somatostatin receptor type 2 gene expression in neuroblastoma, measured by competitive RT-PCR, is related to patient survival and to somatostatin receptor imaging by indium -111-pentetreotide. Medical and pediatric oncology. 2001;36(1):224-6.
54. Tesson M, Vasan R, Hock A, Nixon C, Rae C, Gaze M, et al. An evaluation in vitro of the efficacy of nutlin-3 and topotecan in combination with (177)Lu-DOTATATE for the treatment of neuroblastoma. Oncotarget. 2018;9(49):29082-96.
55. Strosberg J, El-Haddad G, Wolin E, Hendifar A, Yao J, Chasen B, et al. Phase 3 Trial of (177)Lu-Dotatate for Midgut Neuroendocrine Tumors. The New England journal of medicine. 2017;376(2):125-35.
56. Strosberg J, Wolin E, Chasen B, Kulke M, Bushnell D, Caplin M, et al. Health-Related Quality of Life in Patients With Progressive Midgut Neuroendocrine Tumors Treated With (177)Lu-Dotatate in the Phase III NETTER-1 Trial. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2018;36(25):2578-84.
57. Kwekkeboom DJ, de Herder WW, Kam BL, van Eijck CH, van Essen M, Kooij PP, et al. Treatment with the radiolabeled somatostatin analog [177 Lu-DOTA 0,Tyr3]octreotate: toxicity, efficacy, and survival. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2008;26(13):2124-30.
58. Kwekkeboom DJ, Teunissen JJ, Bakker WH, Kooij PP, de Herder WW, Feelders RA, et al. Radiolabeled somatostatin analog [177Lu-DOTA0,Tyr3]octreotate in patients with endocrine gastroenteropancreatic tumors. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2005;23(12):2754-62.
59. Bodei L, Cremonesi M, Grana CM, Fazio N, Iodice S, Baio SM, et al. Peptide receptor radionuclide therapy with (1)(7)(7)Lu-DOTATATE: the IEO phase I-II study. Eur J Nucl Med Mol Imaging. 2011;38(12):2125-35.
60. Kratochwil C, Lopez-Benitez R, Mier W, Haufe S, Isermann B, Kauczor HU, et al. Hepatic arterial infusion enhances DOTATOC radiopeptide therapy in patients with neuroendocrine liver metastases. Endocr Relat Cancer. 2011;18(5):595-602.
61. Virgolini I, Patri P, Novotny C, Traub T, Leimer M, Fuger B, et al. Comparative somatostatin receptor scintigraphy using in-111-DOTA-lanreotide and in-111-DOTA-Tyr3-octreotide versus F-18-FDG-PET for evaluation of somatostatin receptor-mediated radionuclide therapy. Ann Oncol. 2001;12 Suppl 2:S41-5.
62. Kratochwil C, Giesel FL, Bruchertseifer F, Mier W, Apostolidis C, Boll R, et al. (2)(1)(3)Bi-DOTATOC receptor-targeted alpha-radionuclide therapy induces remission in neuroendocrine tumours refractory to beta radiation: a first-in-human experience. Eur J Nucl Med Mol Imaging. 2014;41(11):2106-19.
63. Chan HS, Konijnenberg MW, Daniels T, Nysus M, Makvandi M, de Blois E, et al. Improved safety and efficacy of (213)Bi-DOTATATE-targeted alpha therapy of somatostatin receptor-expressing neuroendocrine tumors in mice pre-treated with L-lysine. EJNMMI Res. 2016;6(1):83.
64. Den RB, Lu B. Heat shock protein 90 inhibition: rationale and clinical potential. Ther Adv Med Oncol. 2012;4(4):211-8.

65. Hong DS, Banerji U, Tavana B, George GC, Aaron J, Kurzrock R. Targeting the molecular chaperone heat shock protein 90 (HSP90): Lessons learned and future directions. Cancer Treatment Reviews. 2013;39(4):375-87.
66. Serwetnyk MA, Blagg BSJ. The disruption of protein-protein interactions with co-chaperones and client substrates as a strategy towards Hsp90 inhibition. Acta Pharm Sin B. 2021;11(6):1446-68.
67. Barrott JJ, Haystead TA. Hsp90, an unlikely ally in the war on cancer. FEBS J. 2013;280(6):1381-96.
68. Sidera K, Patsavoudi E. HSP90 inhibitors: current development and potential in cancer therapy. Recent Pat Anticancer Drug Discov. 2014;9(1):1-20.
69. Sanchez J, Carter TR, Cohen MS, Blagg BSJ. Old and New Approaches to Target the Hsp90 Chaperone. Curr Cancer Drug Targets. 2020;20(4):253-70.
70. ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/home.
71. Pillai RN, Fennell DA, Kovcin V, Ciuleanu TE, Ramlau R, Kowalski D, et al. Randomized Phase III Study of Ganetespib, a Heat Shock Protein 90 Inhibitor, With Docetaxel Versus Docetaxel in Advanced Non-Small-Cell Lung Cancer (GALAXY-2). Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2020;38(6):613-22.
72. Felip E, Barlesi F, Besse B, Chu Q, Gandhi L, Kim SW, et al. Phase 2 Study of the HSP-90 Inhibitor AUY922 in Previously Treated and Molecularly Defined Patients with Advanced Non-Small Cell Lung Cancer. J Thorac Oncol. 2018;13(4):576-84.
73. Uno T, Kawai Y, Yamashita S, Oshiumi H, Yoshimura C, Mizutani T, et al. Discovery of 3-Ethyl-4-(3-isopropyl-4-(4-(1-methyl-1 H-pyrazol-4-yl)-1 H-imidazol-1-yl)-1 H-pyrazolo[3,4- b]pyridin-1-yl)benzamide (TAS-116) as a Potent, Selective, and Orally Available HSP90 Inhibitor. J Med Chem. 2019;62(2):531-51.
74. Doi T, Kurokawa Y, Sawaki A, Komatsu Y, Ozaka M, Takahashi T, et al. Efficacy and safety of TAS-116, an oral inhibitor of heat shock protein 90, in patients with metastatic or unresectable gastrointestinal stromal tumour refractory to imatinib, sunitinib and regorafenib: a phase II, single-arm trial. European journal of cancer (Oxford, England : 1990). 2019;121:29-39.
75. Shimomura A, Yamamoto N, Kondo S, Fujiwara Y, Suzuki S, Yanagitani N, et al. First-in-Human Phase I Study of an Oral HSP90 Inhibitor, TAS-116, in Patients with Advanced Solid Tumors. Mol Cancer Ther. 2019;18(3):531-40.
76. Do KT, O'Sullivan Coyne G, Hays JL, Supko JG, Liu SV, Beebe K, et al. Phase 1 study of the HSP90 inhibitor onalespib in combination with AT7519, a pan-CDK inhibitor, in patients with advanced solid tumors. Cancer chemotherapy and pharmacology. 2020;86(6):815-27.
77. Slovin S, Hussain S, Saad F, Garcia J, Picus J, Ferraldeschi R, et al. Pharmacodynamic and Clinical Results from a Phase I/II Study of the HSP90 Inhibitor Onalespib in Combination with Abiraterone Acetate in Prostate Cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2019;25(15):4624-33.
78. Wagner AJ, Agulnik M, Heinrich MC, Mahadevan D, Riedel RF, von Mehren M, et al. Dose-escalation study of a second-generation non-ansamycin HSP90 inhibitor, onalespib (AT13387), in combination with imatinib in patients with metastatic gastrointestinal stromal tumour. European journal of cancer (Oxford, England : 1990). 2016;61:94-101.

79. Do K, Speranza G, Chang LC, Polley EC, Bishop R, Zhu W, et al. Phase I study of the heat shock protein 90 (Hsp90) inhibitor onalespib (AT13387) administered on a daily for 2 consecutive days per week dosing schedule in patients with advanced solid tumors. Investigational new drugs. 2015;33(4):921-30.
80. Shapiro GI, Kwak E, Dezube BJ, Yule M, Ayrton J, Lyons J, et al. First-in-human phase I dose escalation study of a second-generation non-ansamycin HSP90 inhibitor, AT13387, in patients with advanced solid tumors. Clinical cancer research : an official journal of the American Association for Cancer Research. 2015;21(1):87-97.
81. Momand J, Jung D, Wilczynski S, Niland J. The MDM2 gene amplification database. Nucleic Acids Res. 1998;26(15):3453-9.
82. Konopleva M, Martinelli G, Daver N, Papayannidis C, Wei A, Higgins B, et al. MDM2 inhibition: an important step forward in cancer therapy. Leukemia. 2020;34(11):2858-74.
83. Burgess A, Chia KM, Haupt S, Thomas D, Haupt Y, Lim E. Clinical Overview of MDM2/X-Targeted Therapies. Front Oncol. 2016;6:7.
84. Patton JT, Mayo LD, Singhi AD, Gudkov AV, Stark GR, Jackson MW. Levels of HdmX expression dictate the sensitivity of normal and transformed cells to Nutlin-3. Cancer research. 2006;66(6):3169-76.
85. Brown CJ, Quah ST, Jong J, Goh AM, Chiam PC, Khoo KH, et al. Stapled Peptides with Improved Potency and Specificity That Activate p53. ACS Chemical Biology. 2013;8(3):506-12.
86. Xiong Tan B, Brown CJ, Ferrer FJ, Yuen TY, Quah ST, Chan BH, et al. Assessing the Efficacy of Mdm2/Mdm4-Inhibiting Stapled Peptides Using Cellular Thermal Shift Assays. Scientific Reports. 2015;5:12116.
87. Wei SJ, Chee S, Yurlova L, Lane D, Verma C, Brown C, et al. Avoiding drug resistance through extended drug target interfaces: a case for stapled peptides. Oncotarget. 2016;7(22):32232-46.
88. Yuen TY, Brown CJ, Xue Y, Tan YS, Ferrer Gago FJ, Lee XE, et al. Stereoisomerism of stapled peptide inhibitors of the p53-Mdm2 interaction: an assessment of synthetic strategies and activity profiles. Chem Sci. 2019;10(26):6457-66.
89. Kim YW, Grossmann TN, Verdine GL. Synthesis of all-hydrocarbon stapled alpha-helical peptides by ring-closing olefin metathesis. Nat Protoc. 2011;6(6):761-71.
90. Saleh MN, Patel MR, Bauer TM, Goel S, Falchook GS, Shapiro GI, et al. Phase 1 Trial of ALRN-6924, a Dual Inhibitor of MDMX and MDM2, in Patients with Solid Tumors and Lymphomas Bearing Wild-Type TP53. Clinical cancer research : an official journal of the American Association for Cancer Research. 2021.
91. Thi TTH, Suys EJA, Lee JS, Nguyen DH, Park KD, Truong NP. Lipid-Based Nanoparticles in the Clinic and Clinical Trials: From Cancer Nanomedicine to COVID-19 Vaccines. Vaccines (Basel). 2021;9(4).
92. Fang J, Nakamura H, Maeda H. The EPR effect: Unique features of tumor blood vessels for drug delivery, factors involved, and limitations and augmentation of the effect. Adv Drug Deliv Rev. 2011;63(3):136-51.
93. Munster P, Krop IE, LoRusso P, Ma C, Siegel BA, Shields AF, et al. Safety and pharmacokinetics of MM-302, a HER2-targeted antibody-liposomal doxorubicin conjugate, in patients with advanced HER2-positive breast cancer: a phase 1 dose-escalation study. Br J Cancer. 2018;119(9):1086-93.

94. ClinicalTrials.gov. Study of MBP-426 in Patients With Second Line Gastric, Gastroesophageal, or Esophageal Adenocarcinoma [Available from: https://clinicaltrials.gov/ct2/show/NCT00964080.
95. Barenholz Y. Doxil(R)--the first FDA-approved nano-drug: lessons learned. J Control Release. 2012;160(2):117-34.
96. Edwards K, Johnsson M, Karlsson G, Silvander M. Effect of polyethyleneglycol-phospholipids on aggregate structure in preparations of small unilamellar liposomes. Biophys J. 1997;73(1):258-66.
97. Zetterberg MM, Ahlgren S, Agmo Hernández V, Parveen N, Edwards K. Optimization of lipodisk properties by modification of the extent and density of the PEG corona. J Colloid Interface Sci. 2016;484:86-96.
98. Zetterberg MM, Reijmar K, Pränting M, Engström Å, Andersson DI, Edwards K. PEG-stabilized lipid disks as carriers for amphiphilic antimicrobial peptides. Journal of Controlled Release. 2011;156(3):323-8.
99. Ahlgren S, Fondell A, Gedda L, Edwards K. EGF-targeting lipodisks for specific delivery of poorly water-soluble anticancer agents to tumour cells. Rsc Adv. 2017;7(36):22178-86.
100. Ahlgren S, Reijmar K, Edwards K. Targeting lipodisks enable selective delivery of anticancer peptides to tumor cells. Nanomedicine : nanotechnology, biology, and medicine. 2017;13(7):2325-8.
101. Gao J, Xie C, Zhang M, Wei X, Yan Z, Ren Y, et al. RGD-modified lipid disks as drug carriers for tumor targeted drug delivery. Nanoscale. 2016;8(13):7209-16.
102. Zhang W, Sun J, Liu Y, Tao M, Ai X, Su X, et al. PEG-Stabilized Bilayer Nanodisks As Carriers for Doxorubicin Delivery. Mol Pharmaceutics. 2014;11(10):3279-90.
103. Feng C, Zhang H, Chen J, Wang S, Xin Y, Qu Y, et al. Ratiometric co-encapsulation and co-delivery of doxorubicin and paclitaxel by tumor-targeted lipodisks for combination therapy of breast cancer. Int J Pharm. 2019;560:191-204.
104. Maier P, Hartmann L, Wenz F, Herskind C. Cellular Pathways in Response to Ionizing Radiation and Their Targetability for Tumor Radiosensitization. Int J Mol Sci. 2016;17(1).
105. Spiegelberg D, Abramenkovs A, Mortensen ACL, Lundsten S, Nestor M, Stenerlow B. The HSP90 inhibitor Onalespib exerts synergistic anti-cancer effects when combined with radiotherapy: an in vitro and in vivo approach. Sci Rep. 2020;10(1):5923.
106. Spiegelberg D, Dascalu A, Mortensen AC, Abramenkovs A, Kuku G, Nestor M, et al. The novel HSP90 inhibitor AT13387 potentiates radiation effects in squamous cell carcinoma and adenocarcinoma cells. Oncotarget. 2015;6(34):35652-66.
107. Dungey FA, Caldecott KW, Chalmers AJ. Enhanced radiosensitization of human glioma cells by combining inhibition of poly(ADP-ribose) polymerase with inhibition of heat shock protein 90. Mol Cancer Ther. 2009;8(8):2243-54.
108. Fujii Y, Kato T, Kubota N, Fujimori A, Niwa O, Okayasu R. p53 independent radio-sensitization of human lymphoblastoid cell lines by Hsp90 inhibitor 17-allylamino-17-demethoxygeldanamycin. Oncol Rep. 2010;23(1):199-203.
109. Kinzel L, Ernst A, Orth M, Albrecht V, Hennel R, Brix N, et al. A novel HSP90 inhibitor with reduced hepatotoxicity synergizes with radiotherapy to induce apoptosis, abrogate clonogenic survival, and improve tumor control in models of colorectal cancer. Oncotarget. 2016;7(28):43199-219.

110. Lee Y, Sunada S, Hirakawa H, Fujimori A, Nickoloff JA, Okayasu R. TAS-116, a Novel Hsp90 Inhibitor, Selectively Enhances Radiosensitivity of Human Cancer Cells to X-rays and Carbon Ion Radiation. Mol Cancer Ther. 2017;16(1):16-24.
111. Machida H, Nakajima S, Shikano N, Nishio J, Okada S, Asayama M, et al. Heat shock protein 90 inhibitor 17-allylamino-17-demethoxygeldanamycin potentiates the radiation response of tumor cells grown as monolayer cultures and spheroids by inducing apoptosis. Cancer Sci. 2005;96(12):911-7.
112. Zaidi S, McLaughlin M, Bhide SA, Eccles SA, Workman P, Nutting CM, et al. The HSP90 inhibitor NVP-AUY922 radiosensitizes by abrogation of homologous recombination resulting in mitotic entry with unresolved DNA damage. PloS one. 2012;7(4):e35436.
113. Rodemann HP, Dittmann K, Toulany M. Radiation-induced EGFR-signaling and control of DNA-damage repair. Int J Radiat Biol. 2007;83(11-12):781-91.
114. Park SJ, Kostic M, Dyson HJ. Dynamic Interaction of Hsp90 with Its Client Protein p53. J Mol Biol. 2011;411(1):158-73.
115. He MY, Xu SB, Qu ZH, Guo YM, Liu XC, Cong XX, et al. Hsp90beta interacts with MDM2 to suppress p53-dependent senescence during skeletal muscle regeneration. Aging Cell. 2019;18(5):e13003.
116. Lin K, Rockliffe N, Johnson GG, Sherrington PD, Pettitt AR. Hsp90 inhibition has opposing effects on wild-type and mutant p53 and induces p21 expression and cytotoxicity irrespective of p53/ATM status in chronic lymphocytic leukaemia cells. Oncogene. 2008;27(17):2445-55.
117. Hofving T, Sandblom V, Arvidsson Y, Shubbar E, Altiparmak G, Swanpalmer J, et al. 177Lu-octreotate therapy for neuroendocrine tumours is enhanced by Hsp90 inhibition. Endocr Relat Cancer. 2019;26(4):437-49.
118. Cao C, Shinohara ET, Subhawong TK, Geng L, Kim KW, Albert JM, et al. Radiosensitization of lung cancer by nutlin, an inhibitor of murine double minute 2. Mol Cancer Ther. 2006;5(2):411-7.
119. Feng FY, Zhang Y, Kothari V, Evans JR, Jackson WC, Chen W, et al. MDM2 Inhibition Sensitizes Prostate Cancer Cells to Androgen Ablation and Radiotherapy in a p53-Dependent Manner. Neoplasia. 2016;18(4):213-22.
120. Prabakaran PJ, Javaid AM, Swick AD, Werner LR, Nickel KP, Sampene E, et al. Radiosensitization of Adenoid Cystic Carcinoma with MDM2 Inhibition. Clinical cancer research : an official journal of the American Association for Cancer Research. 2017;23(20):6044-53.
121. Schilling D, Duwel M, Molls M, Multhoff G. Radiosensitization of wildtype p53 cancer cells by the MDM2-inhibitor PXN727 is associated with altered heat shock protein 70 (Hsp70) levels. Cell Stress Chaperones. 2013;18(2):183-91.
122. Arya AK, El-Fert A, Devling T, Eccles RM, Aslam MA, Rubbi CP, et al. Nutlin-3, the small-molecule inhibitor of MDM2, promotes senescence and radiosensitises laryngeal carcinoma cells harbouring wild-type p53. Br J Cancer. 2010;103(2):186-95.
123. Mortensen ACL, Morin E, Brown CJ, Lane DP, Nestor M. Enhancing the therapeutic effects of in vitro targeted radionuclide therapy of 3D multicellular tumor spheroids using the novel stapled MDM2/X-p53 antagonist PM2. EJNMMI Res. 2020;10(1):38.
124. Mortensen ACL, Spiegelberg D, Brown CJ, Lane DP, Nestor M. The Stapled Peptide PM2 Stabilizes p53 Levels and Radiosensitizes Wild-Type p53 Cancer Cells. Front Oncol. 2019;9:923.

125. Spiegelberg D, Mortensen AC, Lundsten S, Brown CJ, Lane DP, Nestor M. The MDM2/MDMX-p53 antagonist PM2 radiosensitizes wild-type p53 tumors. Cancer research. 2018.
126. Foucquier J, Guedj M. Analysis of drug combinations: current methodological landscape. Pharmacol Res Perspect. 2015;3(3):e00149.
127. Roell KR, Reif DM, Motsinger-Reif AA. An Introduction to Terminology and Methodology of Chemical Synergy-Perspectives from Across Disciplines. Front Pharmacol. 2017;8:158.
128. Chou TC, Talalay P. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. Adv Enzyme Regul. 1984;22:27-55.
129. Chou TC. Drug combination studies and their synergy quantification using the Chou-Talalay method. Cancer research. 2010;70(2):440-6.
130. Yadav B, Wennerberg K, Aittokallio T, Tang J. Searching for Drug Synergy in Complex Dose-Response Landscapes Using an Interaction Potency Model. Comput Struct Biotechnol J. 2015;13:504-13.
131. Sharma SV, Haber DA, Settleman J. Cell line-based platforms to evaluate the therapeutic efficacy of candidate anticancer agents. Nat Rev Cancer. 2010;10(4):241-53.
132. Pinto B, Henriques AC, Silva PMA, Bousbaa H. Three-Dimensional Spheroids as In Vitro Preclinical Models for Cancer Research. Pharmaceutics. 2020;12(12).
133. American Type Culture Collection (ATCC) [Available from: http://www.atcc.org.
134. Fong EL, Harrington DA, Farach-Carson MC, Yu H. Heralding a new paradigm in 3D tumor modeling. Biomaterials. 2016;108:197-213.
135. Gilazieva Z, Ponomarev A, Rutland C, Rizvanov A, Solovyeva V. Promising Applications of Tumor Spheroids and Organoids for Personalized Medicine. Cancers (Basel). 2020;12(10).
136. Mao S, Pang Y, Liu T, Shao Y, He J, Yang H, et al. Bioprinting of in vitro tumor models for personalized cancer treatment: a review. Biofabrication. 2020;12(4):042001.
137. Leek R, Grimes DR, Harris AL, McIntyre A. Methods: Using Three-Dimensional Culture (Spheroids) as an In Vitro Model of Tumour Hypoxia. Adv Exp Med Biol. 2016;899:167-96.
138. Ruggeri BA, Camp F, Miknyoczki S. Animal models of disease: pre-clinical animal models of cancer and their applications and utility in drug discovery. Biochem Pharmacol. 2014;87(1):150-61.
139. Day CP, Merlino G, Van Dyke T. Preclinical mouse cancer models: a maze of opportunities and challenges. Cell. 2015;163(1):39-53.
140. Zhou H, Kato A, Yasuda H, Odamaki M, Itoh H, Hishida A. The induction of heat shock protein-72 attenuates cisplatin-induced acute renal failure in rats. Pflugers Arch. 2003;446(1):116-24.
141. Komatsuda A, Wakui H, Oyama Y, Imai H, Miura AB, Itoh H, et al. Overexpression of the human 72 kDa heat shock protein in renal tubular cells confers resistance against oxidative injury and cisplatin toxicity. Nephrol Dial Transplant. 1999;14(6):1385-90.
142. Yokoo T, Kitamura M. IL-1beta depresses expression of the 70-kilodalton heat shock protein and sensitizes glomerular cells to oxidant-initiated apoptosis. J Immunol. 1997;159(6):2886-92.

143. Georgantzi K, Tsolakis AV, Stridsberg M, Jakobson A, Christofferson R, Janson ET. Differentiated expression of somatostatin receptor subtypes in experimental models and clinical neuroblastoma. Pediatric blood & cancer. 2011;56(4):584-9.
144. Gains JE, Sebire NJ, Moroz V, Wheatley K, Gaze MN. Immunohistochemical evaluation of molecular radiotherapy target expression in neuroblastoma tissue. Eur J Nucl Med Mol Imaging. 2018;45(3):402-11.
145. Alexander N, Marrano P, Thorner P, Naranjo A, Van Ryn C, Martinez D, et al. Prevalence and Clinical Correlations of Somatostatin Receptor-2 (SSTR2) Expression in Neuroblastoma. J Pediatr Hematol Oncol. 2019;41(3):222-7.
146. Czapiewski P, Kunc M, Gorczynski A, Haybaeck J, Okon K, Reszec J, et al. Frequent expression of somatostatin receptor 2a in olfactory neuroblastomas: a new and distinctive feature. Hum Pathol. 2018;79:144-50.
147. Gains JE, Moroz V, Aldridge MD, Wan S, Wheatley K, Laidler J, et al. A phase IIa trial of molecular radiotherapy with 177-lutetium DOTATATE in children with primary refractory or relapsed high-risk neuroblastoma. Eur J Nucl Med Mol Imaging. 2020;47(10):2348-57.
148. Gains JE, Aldridge MD, Mattoli MV, Bomanji JB, Biassoni L, Shankar A, et al. 68Ga-DOTATATE and 123I-mIBG as imaging biomarkers of disease localisation in metastatic neuroblastoma: implications for molecular radiotherapy. Nucl Med Commun. 2020;41(11):1169-77.
149. Gains JE, Bomanji JB, Fersht NL, Sullivan T, D'Souza D, Sullivan KP, et al. 177Lu-DOTATATE molecular radiotherapy for childhood neuroblastoma. Journal of nuclear medicine : official publication, Society of Nuclear Medicine. 2011;52(7):1041-7.
150. Al-Ghabkari A, Narendran A. In Vitro Characterization of a Potent p53-MDM2 Inhibitor, RG7112 in Neuroblastoma Cancer Cell Lines. Cancer Biother Radiopharm. 2019;34(4):252-7.
151. Arnhold V, Schmelz K, Proba J, Winkler A, Wunschel J, Toedling J, et al. Reactivating TP53 signaling by the novel MDM2 inhibitor DS-3032b as a therapeutic option for high-risk neuroblastoma. Oncotarget. 2018;9(2):2304-19.
152. Chen L, Pastorino F, Berry P, Bonner J, Kirk C, Wood KM, et al. Preclinical evaluation of the first intravenous small molecule MDM2 antagonist alone and in combination with temozolomide in neuroblastoma. Int J Cancer. 2019;144(12):3146-59.
153. Chen L, Rousseau RF, Middleton SA, Nichols GL, Newell DR, Lunec J, et al. Pre-clinical evaluation of the MDM2-p53 antagonist RG7388 alone and in combination with chemotherapy in neuroblastoma. Oncotarget. 2015;6(12):10207-21.
154. Lakoma A, Barbieri E, Agarwal S, Jackson J, Chen Z, Kim Y, et al. The MDM2 small-molecule inhibitor RG7388 leads to potent tumor inhibition in p53 wild-type neuroblastoma. Cell Death Discov. 2015;1.
155. Lu J, Guan S, Zhao Y, Yu Y, Wang Y, Shi Y, et al. Novel MDM2 inhibitor SAR405838 (MI-773) induces p53-mediated apoptosis in neuroblastoma. Oncotarget. 2016;7(50):82757-69.
156. Van Maerken T, Rihani A, Dreidax D, De Clercq S, Yigit N, Marine JC, et al. Functional analysis of the p53 pathway in neuroblastoma cells using the small-molecule MDM2 antagonist nutlin-3. Mol Cancer Ther. 2011;10(6):983-93.
157. Gamble LD, Kees UR, Tweddle DA, Lunec J. MYCN sensitizes neuroblastoma to the MDM2-p53 antagonists Nutlin-3 and MI-63. Oncogene. 2012;31(6):752-63.

158. Corcoran EB, Hanson RN. Imaging EGFR and HER2 by PET and SPECT: a review. Med Res Rev. 2014;34(3):596-643.
159. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal. 2013;6(269):pl1.
160. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012;2(5):401-4.
161. Verhoef JJ, Carpenter JF, Anchordoquy TJ, Schellekens H. Potential induction of anti-PEG antibodies and complement activation toward PEGylated therapeutics. Drug Discov Today. 2014;19(12):1945-52.
162. Abu Lila AS, Kiwada H, Ishida T. The accelerated blood clearance (ABC) phenomenon: clinical challenge and approaches to manage. J Control Release. 2013;172(1):38-47.
163. Chan TG, O'Neill E, Habjan C, Cornelissen B. Combination Strategies to Improve Targeted Radionuclide Therapy. Journal of nuclear medicine : official publication, Society of Nuclear Medicine. 2020;61(11):1544-52.
164. Park B, Yee C, Lee KM. The effect of radiation on the immune response to cancers. Int J Mol Sci. 2014;15(1):927-43.
165. Mbofung RM, McKenzie JA, Malu S, Zhang M, Peng W, Liu C, et al. HSP90 inhibition enhances cancer immunotherapy by upregulating interferon response genes. Nat Commun. 2017;8(1):451.
166. Blagih J, Buck MD, Vousden KH. p53, cancer and the immune response. J Cell Sci. 2020;133(5).
167. Sgouros G. The Case for Dosimetry in Alpha-Emitter Therapy. J Med Imaging Radiat Sci. 2019;50(4 Suppl 1):S45-S6.
168. Ballal S, Yadav MP, Bal C, Sahoo RK, Tripathi M. Broadening horizons with (225)Ac-DOTATATE targeted alpha therapy for gastroenteropancreatic neuroendocrine tumour patients stable or refractory to (177)Lu-DOTATATE PRRT: first clinical experience on the efficacy and safety. Eur J Nucl Med Mol Imaging. 2020;47(4):934-46.
169. Satapathy S, Sood A, Das CK, Kavanal AJ, Mittal BR. Alpha Before Beta: Exceptional Response to First-Line 225Ac-DOTATATE in a Patient of Metastatic Neuroendocrine Tumor With Extensive Skeletal Involvement. Clin Nucl Med. 2021.
170. Yadav MP, Ballal S, Sahoo RK, Bal C. Efficacy and safety of (225)Ac-DOTATATE targeted alpha therapy in metastatic paragangliomas: a pilot study. Eur J Nucl Med Mol Imaging. 2021.