Applications for DNA-encapsulated silver clusters in physics, biology and medicine

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Chapter 7 - Valorization – Next-generation precision oncology

Cancer remains one of the most formidable challenges in modern medicine, characterized by its complex heterogeneity and resistance to conventional treatment modalities. The treatment landscape for cancer has witnessed remarkable advancements in recent decades. Nevertheless, challenges persist on multiple fronts. First and foremost, the intrinsic heterogeneity of cancer cells within a single tumor and across different patients poses a formidable obstacle to achieving consistent and effective therapeutic outcomes. Additionally, the development of drug resistance mechanisms further complicates the management of cancer. Furthermore, the toxicity associated with many traditional cancer treatments often leads to debilitating side effects, diminishing the quality of life for patients. Traditional light-activated therapies have often faced challenges with limited effectiveness, restricting their broader application, especially in common skin cancers like Basal Cell Carcinoma. In all treatment options, the costs can be significant and even prohibitive, and continue to rise as medical science advances. To address these challenges, we presented a novel light-activated treatment for cancer in Chapter 5 of this thesis. This approach harnesses the power of light in conjunction with a photoactivated agent to selectively destroy cancer cells while sparing healthy tissues. In this chapter, we exhibit our commitment to bringing this treatment to market and our strategy for doing so.

7.1 Introduction

The use of DNA to position objects has been a common practice in science in the past decade. However, oligonucleotide-based technology has shown also great potential for applications to positively impact society directly. From treatment methods such as antisense therapeutics to vaccination for COVID-19, the direct application of these molecules to humans have become both vital and common practice.

This thesis explores various applications for fluorescent and non-fluorescent Ag-DNA, and DNA oligonucleotides, in (bio-)physics applications, treatment options in the medical field, and diagnostic techniques. The chapters are largely divided into different applications in detection and/or treatment.

Chapter 2 applies the sensitivity of the optical properties of the metal cluster to the DNA environment to precisely detect hybridization to target sequences. We introduce several methods of detecting binding through a measurable change in fluorescence. These methods are utilized in the context of optimizing antisense treatments for managing severe genetic conditions, and in the detection of specific sequences related to antibiotic resistance in bacteria.

Chapter 3 utilizes the metal cluster itself as a dark quencher for various dipoles in the form of fluorescent organic dyes. The metal exhibits a strong distance dependent interaction which shows particular promise as a ruler that can detect variations on the sub-nanometer scale. The quenching interaction is calibrated with respect to the distance between a precisely positioned fluorescent dye and the metal surface of the cluster, and studies are performed on the interaction through fluorescence intensity and lifetime experiments.

Chapter 4 introduces the Ag-DNA hairpins as ionic sensors that show a significant change in fluorescence dependent on the local chemical environment of the DNA encapsulation. Depending on the ionic strength of the solution, two populations of fluorescent Ag-DNA 'switch on' or 'switch off', with a change in fluorescence that can be easily detected and calibrated. We utilize these sensors to perform measurements in live cells, nuclei, and relevant biological samples such as urine samples.

Chapter 5 exhibits the potential use of Ag-DNA constructs as a powerful new tool in the treatment of cancer. The silver cluster can be particularly toxic to living organisms and cells due to its strong interaction with nucleic acids. By wrapping the cluster suitably, this toxicity can be limited and controlled. The introduction of a light-sensitive interaction allows us to 'open up' the wrapping to trigger toxicity, using a light source to initiate the change. Results show a strong toxic effect on amoeba, cancer cells, and

simple 3D tissue structures, in collaboration with the Leiden University Medical Center (LUMC).

Chapter 6 utilizes bigger DNA structures, though still extremely small on the scale of 10 nanometers, as drug delivery devices by making enclosed DNA packages. The structures can be produced relatively cheaply through a simple annealing process and can contain molecules for a steady release as the structure degrades thermally and through nuclease activity. The method is tested through gel electrophoresis and dialysis with a fluorescein payload, to monitor the containment and release of the payload. We introduce three such structures to exhibit the tunability of the design. Base pair by base pair fine-tuning of the structure allows the release rate to be managed for a specific requirement, in particular in medical applications.

Two of these topics are the subject of patent applications, one in the treatment of cancer (Chapter 5), and another in detection of nucleotide sequences for the purpose of tracking toxins and other pathogens (Chapter 4). The strong triggered toxicity of the Ag-DNA constructs discussed in Chapter 5 are a particularly exciting result, that we are seeking to develop into a viable product as a spin-off (**GenLumina)** from Leiden University. This chapter will detail the relevance and validity of the treatment and its route to market.

7.2 A Light-activated agent for precision oncology

Cancer describes a broad spectrum of diseases characterized by uncontrolled cell growth, posing a large challenge to global health. Cancer cells have the potential to invade or spread to other parts of the body, initiating a chain of events that disrupt the normal functioning of the body. Amidst the various forms of cancer, skin cancer prominently stands out due to its high prevalence and visibility. It emerges in the skin's epithelial cells and presents in various types, each with distinct characteristics. The most common types include basal cell carcinoma, squamous cell carcinoma, and melanoma, with melanoma being notably aggressive and liable to metastasize. The early onset of skin cancer often manifests through alterations in skin appearance, such as new growths or changes in existing moles or freckles. Understanding the dynamics of skin cancer, its early detection, and prompt intervention are pivotal in mitigating its impacts and improving the prognosis for individuals affected by this very common disease.

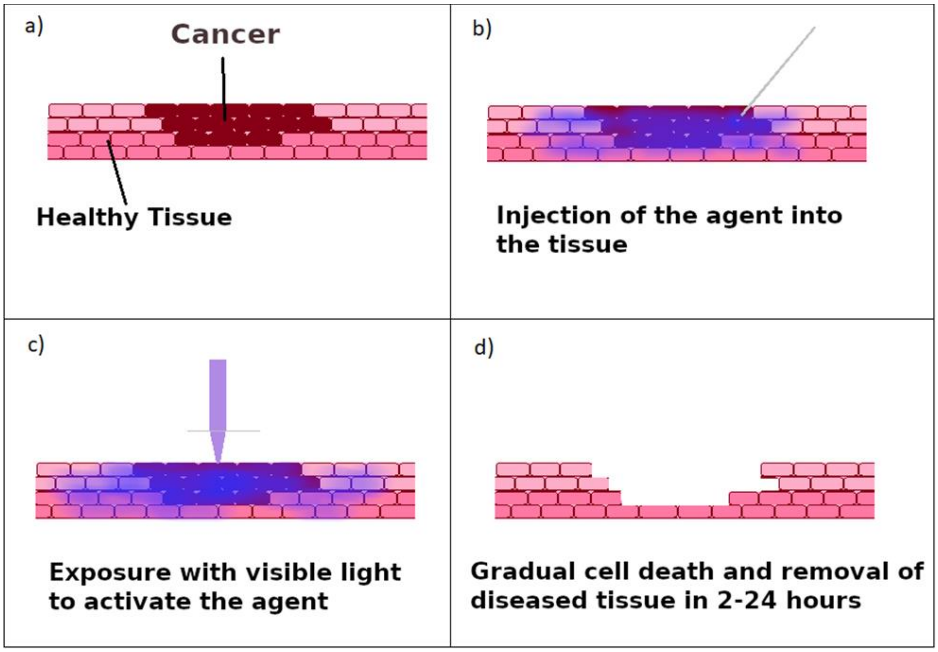

Figure 7.1: Cartoon of the envisioned light-activated cancer treatment. The agent is injected into the tissue region of the tumor, followed by precise activation using a 405 nm laser or other light source. Cell death is induced gradually over the course of 2-24 hours.

The 9C-PC agent introduced in Chapter 5 of this thesis represents an exciting new approach to the treatment of cancer. The agent relies on a specific transition between a toxic and non-toxic state, using an external trigger in the form of light. These 2-3 nanometer constructs are precisely activated using a safe light source, allowing us to target and annihilate cancer cells with remarkable efficacy. Our current testing shows up to 100% toxicity in targeted cells and tissue models, achieving a single-cell level of precision.

The technique should be suitable for the treatment of any cancer type accessible with light. However, Basal Cell Carcinoma (BCC), a form of skin cancer with a very high incidence rate, and accessibility with light, is a particularly suitable initial candidate for the treatment. BCC is the most common form of skin cancer, known for its slow growth and low likelihood of metastasis. However, despite its relatively non-aggressive nature, its prevalence and potential for significant disfigurement necessitate attention and treatment. BCC accounts for approximately 80% of nonmelanoma skin cancers. Its incidence has been increasing globally, partly due to greater public awareness and improved diagnostic methods, but also due to an increasing aging population.

Typically, highly aggressive chemical agents or high-powered lasers can be used for the treatment of cancer. However, the combination of safe light with a safe chemical agent in a design relying on a specific light-matter interaction yields single-cell precision and a high effectiveness of treatment. This new approach to treatment can have reduced recurrence rates and side-effects in patients as a result. Our method can be particularly effective in cases where precision and minimal damage to surrounding tissues are vital, such as in sensitive areas or on the face.

The agent design and application are part of a current Dutch patent application made by Leiden University, with the aim of incorporating the license into a spin-off business we will start within the Leiden Bioscience Park. Appropriate protection of the technology is vital to achieve the level of investment required to bring a treatment such as this to market, a process that will require significant validation and regulatory work over the next 5-10 years.

7.3 The financial landscape for (skin)cancer treatment

The financial ramifications of cancer treatment are, and will continue to be, significant on a global scale. Over the next 30 years, the worldwide cost of cancer is projected to reach a staggering $25 trillion. This financial burden is not evenly distributed, with nations like China, the United States, and India shouldering a significant portion of the costs[86,87]. More specifically, the direct medical costs of cancer care in the United States were nearly $209 billion in 2020 alone[88]. This global economic burden is expected to amount to 0.55% of the annual global gross domestic product between 2020 and 2050, highlighting the substantial economic impact cancer poses[89,90].

Skin cancer, a very prevalent type of cancer, also incurs substantial treatment costs. The cost of treating skin cancer varies significantly based on various factors including the size, type, depth, location of the tumors, and the stage at which the cancer is detected. In the United States, the annual cost for treating skin cancers is estimated at around $8.1 billion, with non-melanoma skin cancers accounting for about $4.8 billion and melanoma accounting for $3.3 billion[91–93]. The total cost of treating skin cancer can range from $400 to $50,000 if one does not have health insurance, and even with insurance, co-payments could range from 10% to 50% of the cost[94]. Globally, the skin cancer treatment market was valued at $9.98 Billion and is anticipated to exceed $18.06 Billion, further demonstrating the financial impact of skin cancer treatments.

These figures show the significant economic burden posed by cancer and skin cancer treatments globally, underlining the need for cost-effective treatment options and

preventative measures. Additionally, the effectiveness and accessibility of treatments often leaves much to be desired. Patients are ever more aware of the options available to them and involved in choosing treatments[95], making the market particularly viable for new, affordable and effective treatment methods.

7.4 Existing treatment options

Skin cancer treatments have evolved significantly, offering a range of options for patients. Surgery is often the first-line treatment for many types of skin cancer, where the cancerous tissue is excised. For basal and squamous cell carcinomas, Mohs surgery is particularly effective[96], involving the removal of the cancer layer by layer and examining it under a microscope until no abnormal cells remain. Radiation therapy is another option, especially useful when surgery isn't feasible, such as in older patients with less effective wound healing. It involves the use of high-energy particles or waves to destroy or damage cancer cells. Using high powered lasers to remove cancer tissue[51,80,81], faces limitations due to the scarcity of trained surgeons and expensive equipment.

Chemotherapy, using chemical compounds to kill cancer cells or inhibit tumor growth, can be systemic or topical. Topical chemotherapy, such as Efudix, is applied to the skin in cases of superficial cancers, while systemic chemotherapy is used for advanced or metastatic skin cancers. In the treatment of well-defined skin tumors, the problem with topical applications in particular is the aggressive nature of the compounds, causing it to be unbearable to patients to the point where it is not used.

Immunotherapy[48,49], a newer range of treatments, harnesses the body's immune system to fight cancer. Drugs like checkpoint inhibitors boost the immune response against cancer cells. Targeted therapy, another advanced treatment, involves drugs that target specific vulnerabilities in cancer cells. These therapies are particularly useful for cancers with certain genetic mutations.

However, these treatments have limitations. Surgery and radiation can cause scarring and other side effects. Chemotherapy often comes with systemic side effects like nausea, fatigue, and hair loss. Immunotherapy and targeted therapy, while promising, are effective only in certain patients and can be expensive. Additionally, there is always the risk of recurrence and the need for ongoing monitoring, making skin cancer a challenging disease to manage.

Light-activated treatments fall under the scope of Photodynamic Therapy (PDT), a medical procedure employed for the treatment of skin conditions. A photosensitizing agent, typically Metvix, is applied topically to the affected area. After a sufficient incubation period, a specific wavelength of light is directed onto the region of the lesion. This activates the Metvix, causing the release of reactive oxygen species, ultimately leading to the destruction of targeted abnormal cells. PDT offers minimally invasive approach, with reduced scarring compared to surgical options. However, it lacks precision and a high enough effectiveness to be a suitable treatment for skin cancer, meaning it is often limited to treating precursors such as Actinic Keratosis. Our treatment method, with a much sharper transition to the toxic state, can have a significantly higher precision and effectiveness to bridge this gap.

7.5 Development and the route to market

Given the early stage of development in our treatment, before the preclinical stage, significant research and development will be required in the short term. In particular, toxicity screening is vital to assess the likelihood of the treatment being allowable in patients. To that end, we have commercial partners in the Leiden Bioscience Park (Toxys B.V.) who specialize in both widespread and in-depth toxicity screening on a cellular level, to expand our knowledge on this matter. Initial in vivo studies will be undertaken in collaboration with the National Cancer Institute (NKI), and we have several commercial alternative options.

In particular, we will initially aim to investigate if all of the silver used in the agent is safely excreted by an organism, or builds up in its organs as well as any other effect on its health. Local studies on the skin after injection will be performed to observe any significant inflammation or other reactions that may be problematic for patients.

In terms of the effectiveness of the agent, challenges will be in the delivery of both the agent and the light to a more complex tissue. We have suitable partners at the LUMC to supply us with full thickness skin models, disease models, and donor material for further testing. The agent's structure allows for optimization to improve the effectiveness and there is a wide scope for changing the dosage chemically on in terms of the light.

Our fledgling business will seek to build a convincing case for bringing the treatment to human testing and, eventually, to market.

7.6 Business structure and revenue model

Those involved in the writing of this thesis will be running this business venture to bring the treatment method to market, utilizing the knowledge gained over the course of our research on the material and treatment method. In particular, cutting-edge technology for the detection of cancer cells can be combined with the treatment method to produce a single, potentially automated, platform for the treatment of skin cancer.

Given the requirement of approval and a long process of testing for the treatment to be allowed onto the market, it is important to identify ways that we can generate revenue and/or research and development funds. Additionally, the quickest and most effective way of obtaining a finalized treatment that can be approved is a priority. We therefore identify an initial business model with three phases in the short, medium and long term.

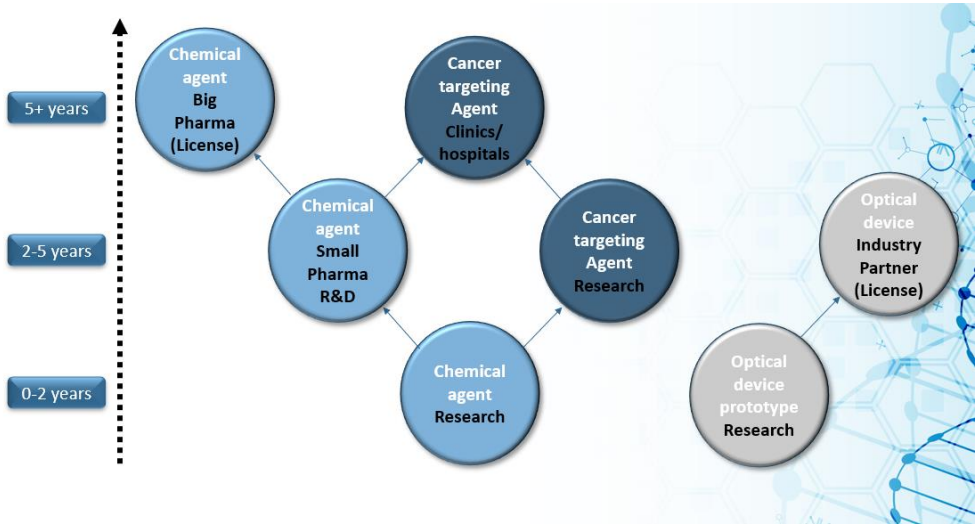

Figure 7.2: Initial description of the GenLumina business model. Given the long route to market, generating revenue and funding for continued research and development is an important aim. Initially (0-2 years), we will focus on subsidies in Public-Private partnerships, supplemented with loans. In the medium term (2-5 years), we will have developed a strong basis for the treatment, including the combination with cancer targeting compounds, to enter the commercial space. We will aim to develop and market the treatment in the longer term (5+ years).

1) Initial Phase (0-2 years): Collaborative Research and Prototype Development

The initial two years prioritize foundational work and collaboration. The start-up aims to engage with cancer marker and imaging research groups to explore joint funding opportunities. Two potential avenues are considered: the start-up acting as a private entity in a collaborative grant application or contributing as a research group in a more academic-focused application process.

One of the central tasks of this phase is to identify appropriate cancer markers that align with the treatment mechanism. This process can also yield a combined IP for a cancer targeting agent that integrates both the treatment methodology and the identified cancer marker.

Additionally, a suitable optical device prototype will be produced for efficient activation of the agent, at least in the research stage.

2) Intermediate Phase (2-5 years): Preclinical and clinical testing

Upon establishing foundational research and securing further IP, the start-up's focus is anticipated to shift towards broader engagement. The experimental results and IP position will significantly de-risk our position as a business, opening the route to more significant investment.

An essential objective during this timeframe is the progression towards clinical trials. This transition is crucial for evaluating the proposed treatment's potential in real-world applications, demanding both rigorous assessment and regulatory compliance, and significant investment into General Manufacturing Practices (GMP) setup.

3) Final Phase (5+ years): Commercialization Strategies

In the longer term, after we obtain approval of the treatment for use in humans, the start-up plans to address the logistics of introducing the treatment to the broader medical community. Two primary strategies are under consideration: the direct market introduction of the treatment or its licensing to established pharmaceutical entities.

For a medical treatment to gain traction and find widespread application, it is crucial that it is observed and trusted as a credible treatment. We must therefore foster contacts with hospital staff and medical professionals who can be important ambassadors for our treatment. Patient organizations are also more well informed and powerful than ever, and can be a vital tool in bringing a novel treatment to being used. Financially, insurance coverage will be vital, which means the cost-effectiveness of the overall treatment has to be significant.

Regarding the light activation technology, a licensing approach is currently favoured. Manufacturers with the capacity to produce either standalone devices or components integrated into existing imaging systems are the intended audience.

In summary, the start-up's structured approach reflects a commitment to thorough research, collaboration, and methodical planning, all with the overarching objective of developing a viable light-activated cancer treatment that can go to market.

7.7 Risk Management

We are in early stages of development in the context of what is required to bring a treatment to market. However, this means we are in a phase requiring modest funding, and as a start-up, are strongly positioned to flexibly and cost-effectively perform pre-clinical studies.

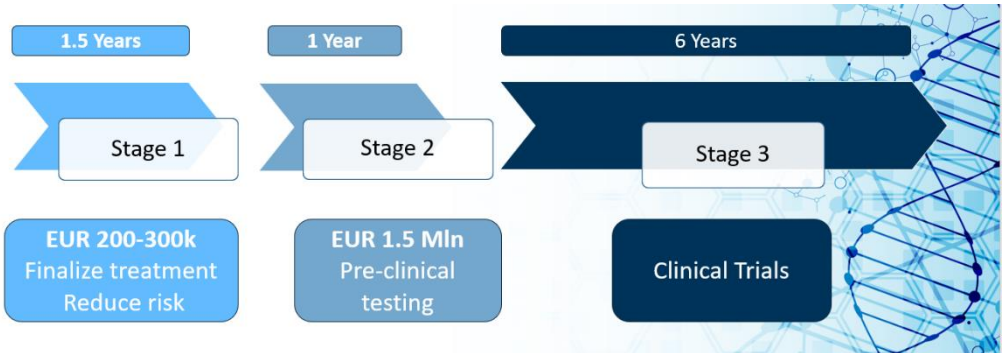

Figure 7.3: GenLumina Budget plan. The initial aim (Stage 1) is to de-risk the proposition by producing convincing initial results in vivo and finalizing the treatment method. This will enable us to obtain suitable investment for final preclinical testing (Stage 2), with wider in vivo studies and GMP setup. In Stage 2, the dossier will be built for initiation of clinical trials, which will require another significant round of investment.

Currently, we are seeking the right partners and acquiring funding (subsidies and loans) to finalize the treatment and perform initial in vivo studies (Stage 1). These tests will be performed on small groups of animals to limit cost, and allow flexibility in modifying the method where necessary. We will produce a strong dossier of testing and optimization of the treatment to assess and reduce the risk of development. This will give us a suitable basis for proceeding to full in vivo testing and the setting up of GMP production (Stage 2), which will require significant investment. Finally, clinical trials will be required to bring the treatment to market, whereby we will require an additional round of investment, an order of magnitude larger than the previous.

The current stage of the process means a substantial risk in investing in an entirely new technology, making outside investment an unlikely (and undesired) choice of acquiring funding during Stage 1. We will, however, engage suitable partners within the market to rely on existing infrastructure, credibility, and expertise. It is important to fit into the existing market structure and treatment options to produce viable product.

7.7.1 Flexibility of targets and application

The 9C-PC agent can theoretically be applied to any target cell that is desired to be removed. Given the usage of light to activate the agent, skin cancer is the most obvious initial application. Additionally, skin cancer treatment is a continually growing market. This is particularly true for basal and squamous cell carcinoma, due to the limited effectiveness of current surgical treatments and increasing incidence rates. There may also be scope for a combination of conventional surgery to remove most of a tumor, and use of the 9C-PC agent to clean up leftover cancer cells. As a secondary, more easily accessible target Actinic Keratosis, a surface skin condition that can be a precursor to skin cancer, can be a more easily treated initial target to produce convincing results and revenue.

Combining the 9C-PC agent with existing or in-development technology for the targeting of cancer cells is an attractive possibility due to the small size of the agent and its many possible binding sites. Internalizing adhesion molecules can improve the effectiveness of the agent by facilitating active transport into the cancer cells. Additionally, the inclusion of a fluorophore enables us to visualize the cancer cells upon binding for precise activation of the agent. For this purpose, we have initiated a collaboration with various research groups for the joint development of targeting agents, including the Image Guided Surgery group at the LUMC.

7.7.2 Radiotherapy enhancement

Radiotherapy, a fundamental component in the battle against cancer, has been significantly enhanced by the introduction of metal nanoparticles, a technique that amplifies the treatment's effectiveness[97,98]. This method, known as radiotherapy enhancement, employs the distinctive attributes of metal particles to boost the impact of radiation on cancer cells. Metal nanoparticles, such as gold (Au), silver (Ag), and platinum (Pt), are known to accumulate in cancer cells due to the enhanced permeability and retention (EPR) effect.

Upon irradiation, these metal particles generate secondary electrons (general photoelectrons and Auger electrons), which have low energy but high linear energy transfer (LET) characteristics. These electrons can cause localized ionization events, leading to direct DNA damage or the production of reactive oxygen species (ROS) within a short range, effectively enhancing the radiation-induced cytotoxicity at the cellular level. Furthermore, scattering events can increase the effective dosage of the radiation locally to further strengthen the effect.

Typically, gold nanoparticles are used in this research, since they are particularly notable for their biocompatibility, ease of synthesis, and strong X-ray absorption coefficient, making them a promising candidate in this field. Platinum nanoparticles also offer the dual advantage of radiosensitization and direct chemotherapeutic effects. Silver also has this quality, and while currently not significantly researched for this purpose, Ag-DNAs, by virtue of their small size and unique structure, are adept at accumulating in cancer cells. Additionally, the DNA encapsulation lends itself to incorporation of other metals such as gold or platinum.

The efficacy of this technique is supported in initial studies[99] , which showed a significant increase in survival rates in mice with cancer treated with gold nanoparticles and radiation therapy. Despite its promising results, the technique faces challenges such as the delivery and distribution of nanoparticles within tumors, potential toxicity issues, and the need for precise dosimetry to optimize treatment outcomes. Current research is directed towards overcoming these challenges, developing targeted delivery systems, and examining the synergistic effects with other treatment modalities. Metal-DNA hybrids represent a particularly interesting material for this purpose, due to their biocompatibility and suitability for combination with targeting compounds.

The development of the metal-DNA agent as a radiotherapy enhancer can be a significantly easier route to market, and would be covered by our existing IP, even if the structure and metal have to be modified. It is therefore of particular relevance to investigate this option as a second potential product, as an alternative to the light-activated treatment, to reduce the commercial risk.

7.7.3 Leiden Biotech infrastructure

The work in this thesis was performed at Leiden University, leaving us strongly positioned within the Leiden Bioscience Park. Here, we have a great deal of resources and potential partners available to share some of the risk and expedite the process of development. The LUMC and the National Cancer Institute in Amsterdam are strong

partners ensuring clinical relevance of our treatment, and granting us access to existing treatment methods. Patient advocacy groups are also a vital part of the modern medical landscape, which makes integration into the hospital system vital for marketing our treatment if it proves effective in patients.

Pre-incubation, incubation, and acceleration programs are available through PLNT, Unlock_ and the greater Bioscience Park to grant start-up companies a strong environment with resources to build a viable business. Apart from training and contacts, these programs will enable us to use affordable lab space and other requirements to limit our early budget requirements in the de-risking phase.

7.8 Intellectual Property

The technology is currently subject of a Dutch patent application. It is pending, but initial feedback from the patent office was sufficiently positive to ensure us of having a suitably broad patent to protect the treatment and gives a wide range of application and optimization.

As a spin-off from Leiden University, GenLumina will purchase the unlimited and exclusive license to the patent from the University's holding company (LEH). The original inventors are part of the co-founders and partially hold rights to the proceeds from the patent as well.

In the short term, we will conduct thorough market research to identify and prioritize regions where the technology could have the most significant impact. We will focus on markets with high prevalence of skin cancer and Actinic keratosis, as well as those with advanced healthcare infrastructure, making, for example, the United States a particularly important market.

The IP strategy also plays a role in the continued testing and development process, in the sense that regulatory approval will also be needed, such as CE marking in Europe, and FDA approval in the United States. Identifying the most relevant markets can expedite this process by producing the most convincing experimental results for those markets. IP management will also represent a significant part of the budget needs.

7.9 Conclusions

In this chapter, we share our vision in bringing the technology developed over the course of this thesis work to market. Apart from satisfying curiosity, we believe it is important for scientific results to be relevant to society and address real world problems. The only way to do so is to produce a viable business case and fit into the existing market landscape.

We believe treatment methods based on metal-DNA technology can be an exciting new approach to add to the next generation of cancer treatments, with high effectiveness, precision, and affordable cost. The treatment of cancer is one of the largest costs to society both in lives and financially, and will continue to be a growing challenge over the next decades.

However, bringing a medical treatment to market is a costly, complicated, and long process, in particular in the field of oncology. Because of this, a thorough strategy to manage risk and seek cost effectiveness in research is vital. A strong support from medical professionals and treatment providers will be required throughout the process, and we are building a strong community of knowledge and resources to make our treatment a success. We encourage anyone to reach out to us and join us in our endeavors.