Oncolytic virotherapy - analysis, design, models

Bhatt, Darshak

DOI:
10.33612/diss.859671389

Appendix

English summary

Typically, viruses are infectious agents responsible for causing a multitude of diseases, ranging from the common cold to severe pandemics such as HIV and COVID-19. However, scientific insights and technological advances in the field of genetic engineering have allowed us to harness these viruses for the treatment of cancer. Such oncolytic (cancer-killing) viruses are the focal point of research in this present thesis. Certainly, the use of viruses as a cancer treatment requires them to be safe i.e., not cause adverse symptoms in patients, and simultaneously be effective in reducing tumor burden and improving patient survival.

Through an interdisciplinary approach, as in this thesis, we studied strategies focused on improving the safety and efficacy of oncolytic virotherapy.

The thesis begins by considering the counterintuitive idea of employing viruses to treat cancer and provides a brief insight into the historical milestones in the development of oncolytic viruses (**Chapter 1**). Active research since the 1940s has established that oncolytic viruses act by infecting and killing cancer cells, followed by activation of the patient's immune system to eliminate cancer. Advances in the field of genetic engineering have allowed researchers to modify viruses for example by 'deletion' of pathogenic factors or by engineering the virus to specifically 'target' and/or 'replicate' in cancer cells. Various natural and modified viruses have been considered as potential therapeutic candidates and are currently undergoing clinical trials to determine safety and efficacy. A prime example of oncolytic virotherapy is Imlygic or Talimogene laherparepvec (T-VEC), a genetically modified herpes virus-based therapy that gained regulatory approval for melanoma treatment.

In **Chapter 2**, we evaluate the clinical success of virus candidates used as cancer therapeutics. We access published literature to learn about the trial design, the nature of viruses and related genetic modifications, patient background, and the assessment of clinical outcomes related to therapeutic safety and efficacy. Our findings demonstrate that oncolytic virotherapy is, for most of the virus candidates, safe, with manageable side effects such as fever and fatigue. However, it is crucial to note that this approach is still in its early stages of clinical testing, and further rigorous evaluation is required through randomized trial design, particularly involving diverse patient groups. It is also important to assess its compatibility with conventional cancer treatments.

As a focus of this thesis, we design an oncolytic virus based on Semliki Forest virus. We use a safe version of Semliki Forest virus, called recombinant replicon particles. These recombinant Semliki Forest virus particles are capable of a single round of infection in target cancer cells and do not produce viral progeny, thus maintaining a safe profile by limiting virus spread to healthy tissue. To enhance efficacy, we modify the virus genome to produce immunostimulatory cytokine signals upon infection in target cancer cells. These cytokines play a pivotal role in

promoting immune cell recruitment and activation in the tumor. **Chapter 3** is an experimental study that explores the potential of recombinant Semliki Forest virus to enhance immune responses within the tumor microenvironment. We find that even without the production of virus-encoded cytokines, infected cancer cells have an inherent although basic ability to trigger robust immune responses. Our study further highlights the potential of encoding specific cytokines to amplify these immune responses, offering a promising avenue for boosting the immune system's cancer-fighting capabilities.

Cancer cells exist in a complex and dynamic environment and continuously interact with other healthy stromal cells, immune cells, blood vessels, extracellular matrix, and soluble factors. In **Chapter 4**, we shift the focus to a vital component of the tumor microenvironment – tumor-derived extracellular vesicles. Extracellular vesicles are minuscule, bilipid-layered, droplet-like entities, that contain various biochemical signals such as DNA, RNA, proteins, and metabolites. Here, we review existing literature to understand how extracellular vesicles released by cancer cells are involved in communication with various cells in the tumor microenvironment. We discuss how these extracellular vesicles influence tumor progression and ultimately therapy response by influencing tumor growth, formation of blood vessels, cancer metastasis, and the creation of an immunosuppressive environment. Importantly, we elaborate on how these characteristic features of tumor-derived extracellular vesicles may perhaps be exploited for diagnostics or prognostic purposes.

Building on the understanding of tumor extracellular vesicles, **chapter 5** examines how oncolytic virotherapy impacts these vesicles. The study involves characterizing the physical and biochemical features of tumor extracellular vesicles released from cancer cells treated with recombinant Semliki Forest virus replicon particles. Our findings reveal a novel mechanism through which virotherapy can enhance anti-tumor immunity. We show that tumor extracellular vesicles secreted by melanoma cells suppress the activation of immune cells. However, oncolytic virus infection alters the characteristics and functions of tumor extracellular vesicles, making them more conducive to triggering an immunogenic response. These results suggest that oncolytic virotherapy minimizes the immune-suppressive effects of tumor extracellular vesicles.

Tumors manifest various resistance mechanisms to escape oncolytic virotherapy. **Chapter 6**, conducts a systematic analysis of existing literature to compile a detailed understanding of the various ways in which the tumor can resist oncolytic virotherapy. We categorize these mechanisms into tumor-cell mediated, stromal cell-mediated, immune responses, and systemic responses. Our analysis indicates that oncolytic virotherapy may fail not only due to resistance of cancer cells but also due to stromal and immune cells acting locally or systemically in the tumor microenvironment. The chapter aids in understanding these resistance mechanisms to overcome them and develop more effective virotherapy strategies.

We next perform a computational study to find strategies to overcome resistance mediated by cancer and stromal cells in a spatiotemporal context. In **Chapter 7**, we design a computational model that allows us to study virus-cell interaction. The model reveals that factors like virus replication parameters, the spatial architecture of the tumor, and the presence of resistant cancer and stromal cells can significantly influence the therapy's effectiveness. The findings point to strategies to enhance the therapy, such as improving virus dispersal within the tumor or sensitizing stromal cells without harming healthy tissues.

Chapter 8 builds upon the computational model by focusing on T cell-mediated immune responses. We upgrade the model to include the effects of antiviral or anticancer-specific T cell cytotoxicity. The study explores how T cell cytotoxicity, tumor density, and the dynamics of inflammatory molecules affect treatment outcomes. Our findings demonstrate that an effective immune response results from a complex interplay between these factors and can either enhance or hinder the success of oncolytic virotherapy. We observe that, ideally, immune responses aid total tumor eradication only when virotherapy induces the release of potent inflammatory molecules that diffuse rapidly in the tumor microenvironment.

In **Chapter 9** we take a step back to explore the broader landscape of genetic engineering and synthetic biology in the Netherlands. This chapter highlights the growing interest in synthetic biology in Dutch academia and industry and emphasizes the need for greater collaboration and long-term partnerships to advance the field. To this end, we highlight the work done by SynBioNL, the association of synthetic biology in the Netherlands, aiming to bridge the stakeholders. This growing community of synthetic biologists in the Netherlands holds a promise to reach various goals including improving health, nutrition, and economy.

The thesis culminates in a general discussion that synthesizes the key findings from the various chapters (**Chapter 10**). We reflect on the potential of oncolytic virotherapy based on Semliki Forest virus and provide a perspective on future developments, including improved controllability and efficacy.

With this thesis we hope to contribute to the theoretical understanding of oncolytic virotherapy and that we have also contributed to practical insights that can lead to the development of new, effective cancer treatments.

Nederlandse samenvatting

Virusinfecties kunnen ziekten veroorzaken die kunnen variëren van een gewone verkoudheid tot ernstige ziekten en pandemieën zoals HIV, Ebola en COVID-19. Echter, wetenschappelijke onderzoek en technologische vooruitgang op het gebied van genetische engineering hebben ons in staat gesteld om virussen ook te gebruiken voor de behandeling van kanker. Het gebruik van zulke oncolytische (kanker-dodende) virussen voor kankerpatiënten vereist natuurlijk dat ze veilig zijn, en tegelijkertijd dat ze effectief zijn voor de behandeling van kanker. Deze aspecten staan centraal in het onderzoek beschreven in dit proefschrift.

Het proefschrift begint met het bespreken van een verrassend idee: het gebruik van virussen om kanker te behandelen. Het geeft een kort overzicht van belangrijke gebeurtenissen in de geschiedenis van de ontwikkeling van virussen voor dit doel (**Hoofdstuk 1**). Onderzoek sinds de jaren 1940 heeft aangetoond dat sommige virussen kankercellen kunnen binnen dringen en doden. Daarna wordt het immuunsysteem van de patiënt ingeschakeld om de resterende kankercellen te doden. Dankzij vooruitgang in genetische technologie kunnen wetenschappers virussen aanpassen, bijvoorbeeld door schadelijke delen te verwijderen of ze specifiek te laten richten op, en vermenigvuldigen in, kankercellen. De veiligheid en effectiviteit van verschillende natuurlijke en aangepaste virussen zijn inmiddels onderzocht in klinische studies. Een voorbeeld van zo'n behandeling is Imlygic of Talimogene laherparepvec (T-VEC), een therapie gebaseerd op een gemodificeerd herpesvirus. Deze therapie is goedgekeurd voor de behandeling van melanoom.

In **Hoofdstuk 2** beschrijven we een analyse van het klinische succes van oncolytische virotherapie in patiënten. We inventariseerden gepubliceerde literatuur naar de opzet van studies met oncolytische virussen, de gebruikte virussen, -al dan niet genetisch gemodificeerd-, en de klinische resultaten met betrekking tot de veiligheid en effectiviteit van de behandeling. Deze analyse laat zien dat oncolytische virotherapie voor de meeste viruskandidaten veilig is; met beheersbare bijwerkingen zoals koorts en vermoeidheid. Het is wel belangrijk om te realiseren dat de klinische evaluatie van oncolytische virotherapie zich nog in een relatief vroeg stadium bevindt en er uitgebreide gerandomiseerde studies in patiënten met verschillende vormen van kanker noodzakelijk zijn om de daadwerkelijke effectiviteit van deze behandeling te beoordelen.

De focus van dit proefschrift richt zich daarna op de ontwikkeling van oncolytische virotherapie gebaseerd op het Semliki Forest virus (SFV). We gebruiken een veilige versie van het virus, zogenaamde SFV replicon-deeltjes. Deze SFV replicon-deeltjes zijn in staat tot één infectieronde in (kanker) cellen en produceren geen nieuwe virussen. Dit is een eerste waarborg voor veiligheid. Om een sterke afweerreactie op te bouwen, modificeren we het virus RNA zodanig dat het ook codeert voor bepaalde cytokinen die afweercellen kunnen aantrekken

en/of activeren. Cellen, geïnfecteerd met deze SFV replicon-deeltjes, produceren na infectie deze cytokines en kunnen hiermee de afweerreactie verder versterken.

Hoofdstuk 3 is een experimentele studie naar de potentie van recombinant SFV om immuunreacties binnen de tumoromgeving op te wekken. We ontdekken dat SFV replicon-deeltjes zelfs zonder cytokine-modificatie al een krachtige immuunreacties veroorzaken. We laten verder zien dat expressie van de cytokines deze afweerreactie verder kan versterken en hiermee dat behandeling met SFV replicon-deeltjes veelbelovend kan zijn en de moeite waard om verder te ontwikkelen voor oncolytische virotherapie.

In **Hoofdstuk 4** verschuiven we de focus naar een specifiek aspect van tumoren, de door kankercellen afgescheiden extracellulaire vesicles. Extracellulaire vesicles zijn kleine vet/eiwit-bolletjes die verschillende biochemische signaalmoleculen kunnen bevatten, zoals DNA, RNA, eiwitten en metabolieten. Het is goed om te realiseren dat tumoren naast kankercellen ook andere cellen en weefsels, zoals stromale cellen, afweercellen, bloedvaten, extracellulaire matrix, etc. kunnen bevatten. Daarom onderzochten we, zoals beschreven in hoofdstuk 4, de wetenschappelijke literatuur om in kaart te brengen hoe, door kankercellen uitgestoten, extracellulaire vesicles betrokken zijn bij de communicatie met verschillende cellen in de tumoromgeving. We analyseerden hoe extracellulaire vesicles tumorprogressie beïnvloeden en de reactie op therapie beïnvloeden door invloed uit te oefenen op tumorgroei, de vorming van bloedvaten, uitzaaiingen en het induceren van een afweer onderdrukkende omgeving. We gaan ook in op hoe bepaling van de eigenschappen van de verschillende extracellulaire vesicles mogelijk kan worden benut voor diagnostische of prognostische doeleinden.

Voortbouwend op hoofdstuk 4 onderzochten we hoe oncolytische virotherapie deze vesicles beïnvloedt. Deze studie (**Hoofdstuk 5**) omvat het karakteriseren van de fysieke en biochemische kenmerken van tumor extracellulaire vesicles die worden afgegeven door kankercellen behandeld met SFV replicon-deeltjes. We tonen aan dat extracellulaire vesicles afgescheiden door melanoomcellen de activatie van afweercellen onderdrukken. Echter, infectie met SFV replicon-deeltjes verandert de kenmerken en functies van deze vesicles, waardoor ze wel een afweer respons opwekken. Deze studie suggereert dat oncolytische virotherapie gebaseerd op SFV de immuun onderdrukkende effecten van tumor extracellulaire vesicles kan verminderen.

Tumoren hebben verschillende mechanismen om te ontsnappen aan oncolytische virotherapie. Om dit beter te begrijpen voerden we een systematische analyse uit van de wetenschappelijke literatuur naar de verschillende manieren waarop de tumor resistent kan zijn/worden voor oncolytische virotherapie (**Hoofdstuk 6**). We categoriseren deze mechanismen in i) door tumorcellen gemedieerde responsen, ii) door stromale cellen gemedieerde responsen, iii) immuun responsen en iv) systemische responsen. Onze analyse laat zien dat oncolytische virotherapie kan falen door resistentie van kankercellen, maar ook door stromale cellen en/of immuun cellen in de tumor. Het hoofdstuk helpt bij het begrijpen van

deze resistentiemechanismen wat kan helpen ze te overwinnen om effectievere virotherapie strategieën te ontwikkelen.

Vervolgens voerden we een modellering studie uit om strategieën te vinden om resistentie, gemedieerd door kanker- en stromale cellen, te overwinnen in een ruimtelijk en temporele context. In **Hoofdstuk 7** beschrijven we een door ons ontworpen model waarmee we de interactie tussen virus en cel kunnen bestuderen. Het model onthult dat factoren zoals virus replicatie parameters, de ruimtelijke architectuur van de tumor en de aanwezigheid van resistente kanker- en stromale cellen een aanzienlijke invloed hebben op de effectiviteit van de therapie. De bevindingen wijzen op strategieën om de therapie te verbeteren, zoals het verbeteren van de verspreiding van het virus binnen de tumor of het gevoeliger maken van stromale cellen zonder gezond weefsel te schaden.

Hoofdstuk 8 bouwt voort op het model nu ook gericht op T cel-gemedieerd antikanker afweerreacties. De studie onderzoekt hoe T cel cytotoxiciteit, tumordichtheid en de dynamiek van ontstekingsmoleculen van invloed zijn op behandelingsresultaten. Onze bevindingen laten zien dat een effectieve immuunrespons het resultaat is van een complex samenspel tussen deze factoren en het succes van oncolytische virotherapie zowel kan verbeteren als belemmeren. Deze modelleringstudie suggereert dat idealiter, afweerreacties alleen leiden tot totale tumor destructie wanneer virotherapie de afgifte van krachtige ontstekingsmoleculen opwekt die snel diffunderen in de tumoromgeving.

Hoofdstuk 9 verkent het bredere landschap van genetische engineering en synthetische biologie in Nederland. Dit hoofdstuk belicht de en mijn groeiende interesse in synthetische biologie in de Nederlandse academische wereld en industrie en benadrukt de noodzaak van meer samenwerking en lange termijn partnerschappen om het vakgebied vooruit te helpen. In dit kader benadrukken we het werk van SynBioNL, de vereniging voor synthetische biologie in Nederland, die als doel heeft belanghebbenden met elkaar te verbinden. Deze groeiende gemeenschap van synthetische biologen in Nederland belooft verschillende doelen te bereiken, waaronder het verbeteren van gezondheid, voeding en economie.

Het proefschrift wordt afgesloten met een algemene discussie die de belangrijkste bevindingen uit de verschillende hoofdstukken synthetiseert (**Hoofdstuk 10**). We reflecteren op het potentieel van oncolytische virotherapie gebaseerd op SFV en bieden een perspectief op toekomstige ontwikkelingen, waaronder verbeterde controleerbaarheid en doeltreffendheid.

We hopen met dit proefschrift bij te dragen aan het theoretisch begrip van oncolytische virotherapie en dat we daarnaast bijgedragen hebben aan praktische inzichten die kunnen leiden tot de ontwikkeling van nieuwe, effectieve kankerbehandelingen.

Resumo em Português

Normalmente, os vírus são agentes infecciosos responsáveis por causar uma infinidade de doenças, desde o resfriado comum até pandemias graves, como HIV e COVID-19. Contudo, os conhecimentos científicos e os avanços tecnológicos no campo da engenharia genética permitiram-nos utilizar estes vírus para o tratamento do câncer. Esses vírus oncolíticos (que eliminam o câncer) são o ponto focal da pesquisa nesta presente tese. Certamente, a utilização de vírus como tratamento do câncer exige que estes sejam seguros, isto é, não causem efeitosadversos nos pacientes e, simultaneamente, sejam eficazes na redução da carga tumoral e na melhoria da sobrevivência dos pacientes.

Através de uma abordagem interdisciplinar, como nesta tese, estudamos estratégias focadas em melhorar a segurança e eficácia da viroterapia oncolítica.

A tese começa considerando a ideia contra-intuitiva de empregar vírus para tratar o câncer e fornece uma breve visão dos marcos históricos no desenvolvimento de vírus oncolíticos (**Capítulo 1**). Pesquisas ativas desde a década de 1940 estabeleceram que os vírus oncolíticos agem infectando e matando células cancerígenas, seguido pela ativação do sistema imunológico do paciente para eliminar o câncer. Os avanços no campo da engenharia genética permitiram aos investigadores modificar os vírus, por exemplo, através da "eliminação" de fatores patogênicos ou da engenharia do vírus para infectar e/ou "replicar-se" especificamente em células cancerígenas. Vários vírus naturais e modificados foram considerados potenciais candidatos terapêuticos e estão atualmente em ensaios clínicos para determinar a segurança e eficácia. Um excelente exemplo de viroterapia oncolítica é Imlygic ou Talimogene laherparepvec (T-VEC), uma terapia baseada no vírus do herpes geneticamente modificado que obteve aprovação regulatória para o tratamento do melanoma.

No **Capítulo 2**, avaliamos o sucesso clínico de candidatos virais utilizados como terapia contra o câncer. Acessamos a literatura publicada para aprender sobre o desenho do ensaio, a natureza dos vírus e modificações genéticas relacionadas, o histórico do paciente e a avaliação dos resultados clínicos relacionados à segurança e eficácia terapêutica. Nossas descobertas demonstram que a viroterapia oncolítica é, para a maioria dos candidatos a vírus, segura, com efeitos colaterais controláveis, como febre e fadiga. No entanto, é crucial notar que esta abordagem ainda está nos seus estágios iniciais de testes clínicos, e é necessária uma avaliação mais rigorosa através de um desenho de ensaios randomizados, particularmente envolvendo diversos grupos de pacientes. Também é importante avaliar a sua compatibilidade com os tratamentos convencionais contra o câncer.

Como foco desta tese, projetamos um vírus oncolítico baseado no vírus Semliki Forest. Usamos uma versão segura do vírus Semliki Forest, chamada de partículas de replicon recombinantes. Estas partículas recombinantes do vírus Semliki Forest são capazes de um único ciclo de infecção em células cancerígenas alvo e não

produzem descendência viral, mantendo assim um perfil seguro ao limitar a propagação do vírus a tecidos saudáveis. Para aumentar a eficácia, modificamos o genoma do vírus para produzir citocinas imune-estimulantes após infecção em células cancerígenas alvo. Essas citocinas desempenham um papel fundamental na promoção do recrutamento e ativação de células imunológicas no tumor. O **Capítulo 3** é um estudo experimental que explora o potencial do vírus recombinante Semliki Forest para melhorar as respostas imunológicas dentro do microambiente tumoral. Descobrimos que mesmo sem a produção de citocinas codificadas por vírus, as células cancerígenas infectadas têm uma capacidade inerente, embora básica, de desencadear respostas imune robustas. Nosso estudo destaca ainda o potencial de codificação de citocinas específicas para amplificar essas respostas imunológicas, oferecendo um caminho promissor para aumentar as capacidades do sistema imunológico de combate ao câncer.

As células cancerosas existem em um ambiente complexo e dinâmico e interagem continuamente com outras células estromais saudáveis, células do sistema imunológico, vasos sanguíneos, matriz extracelular e fatores solúveis. No **Capítulo 4**, mudamos o foco para um componente vital do microambiente tumoral – as vesículas extracelulares derivadas do tumor. As vesículas extracelulares são entidades nanométricas, com uma bicamada lipídica e semelhantes a gotículas, que contêm vários sinais bioquímicos, como DNA, RNA, proteínas e metabólitos. Aqui, revisamos a literatura existente para entender como as vesículas extracelulares liberadas pelas células cancerígenas estão envolvidas na comunicação com várias células no microambiente tumoral. Discutimos como essas vesículas extracelulares influenciam a progressão do tumor e, em última análise, a resposta à terapia, influenciando o crescimento do tumor, a formação de vasos sanguíneos, a metástase do câncer e a criação de um ambiente imunossupressor. É importante ressaltar que elaboramos como essas características das vesículas extracelulares derivadas de tumores podem talvez ser exploradas para fins diagnósticos ou prognósticos.

Com base na compreensão das vesículas extracelulares tumorais, o **capítulo 5** examina como a viroterapia oncolítica afeta essas vesículas. O estudo envolve a caracterização física e bioquímica das vesículas extracelulares tumorais liberadas de células cancerígenas tratadas com partículas de replicon do vírus Semliki Forest recombinantes. Nossas descobertas revelam um novo mecanismo através do qual a viroterapia pode aumentar a imunidade antitumoral. Mostramos que as vesículas extracelulares tumorais secretadas pelas células do melanoma suprimem a ativação das células imunológicas. Contudo, a infecção por vírus oncolíticos altera as características e funções das vesículas extracelulares tumorais, tornando-as mais propícias ao desencadeamento de uma resposta imunogênica. Estes resultados sugerem que a viroterapia oncolítica minimiza os efeitos imunossupressores das vesículas extracelulares tumorais.

Os tumores manifestam vários mecanismos de resistência para escapar da viroterapia oncolítica. O **Capítulo 6** conduz uma análise sistemática da literatura

existente para compilar uma compreensão detalhada das várias maneiras pelas quais o tumor pode resistir à viroterapia oncolítica. Nós categorizamos esses mecanismos em respostas imunes mediadas por células tumorais, mediadas por células estromais e respostas sistêmicas. Nossa análise indica que a viroterapia oncolítica pode falhar não apenas devido à resistência das células cancerosas, mas também devido às células do estroma e do sistema imunológico que atuam local ou sistemicamente no microambiente tumoral. O capítulo auxilia na compreensão desses mecanismos de resistência para superá-los e desenvolver estratégias de viroterapia mais eficazes.

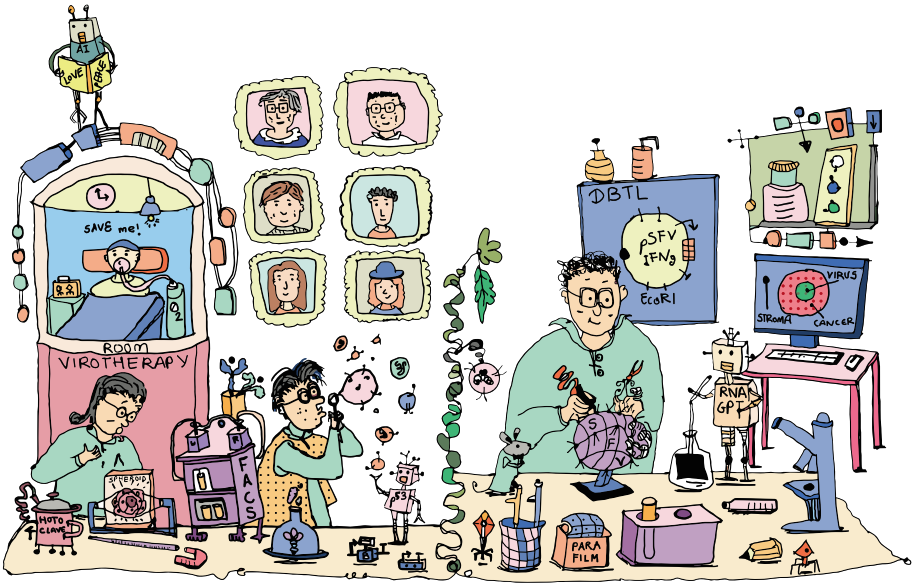
Em seguida, realizamos um estudo computacional para encontrar estratégias para superar a resistência mediada por células cancerígenas e células estromais em um contexto espaço temporal. No **Capítulo 7**, projetamos um modelo computacional que nos permite estudar a interação vírus-célula. O modelo revela que fatores como a replicação do vírus, a arquitetura espacial do tumor e a presença de células cancerígenas resistentes e células estromais podem influenciar significativamente a eficácia da terapia. As descobertas apontam para estratégias para melhorar a terapia, melhorarando a dispersão do vírus dentro do tumor ou sensibilizando as células do estroma sem prejudicar os tecidos saudáveis.

O **Capítulo 8** baseia-se no modelo computacional, concentrando-se nas respostas imunes mediadas por células T. Atualizamos o modelo para incluir os efeitos da citotoxicidade de células T antivirais ou anticâncer específicas. O estudo explora como a citotoxicidade das células T, a densidade do tumor e a dinâmica das moléculas inflamatórias afetam os resultados do tratamento. Nossas descobertas demonstram que uma resposta imune eficaz resulta de uma interação complexa entre esses fatores e pode aumentar ou dificultar o sucesso da viroterapia oncolítica. Observamos que, idealmente, as respostas imunes auxiliam na erradicação total do tumor apenas quando a viroterapia induz a liberação de moléculas inflamatórias potentes que se difundem rapidamente no microambiente tumoral.

No **Capítulo 9** damos um passo atrás para explorar o panorama mais amplo da engenharia genética e da biologia sintética na Holanda. Este capítulo destaca o crescente interesse pela biologia sintética na academia e na indústria holandesa e enfatiza a necessidade de maior colaboração e parcerias de longo prazo para avançar no campo. Para tal, destacamos o trabalho realizado pela SynBioNL, a associação de biologia sintética dos Países Baixos, com o objetivo de aproximar as partes interessadas. Esta comunidade crescente de biólogos sintéticos nos Países Baixos promete alcançar vários objetivos, incluindo a melhoria da saúde, nutrição e economia.

A tese culmina com uma discussão geral que sintetiza as principais conclusões dos vários capítulos (**Capítulo 10**). Reflete sobre o potencial da viroterapia baseada no vírus Semliki Forest e fornece uma perspectiva sobre desenvolvimentos futuros, incluindo melhor controle e eficácia. No geral, a tese

cobre uma ampla gama de tópicos, contribuindo não apenas para a compreensão teórica da viroterapia oncolítica, mas também oferecendo insights práticos focados em novos tratamentos eficazes contra o câncer no futuro.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Acknowledgements

Before a PhD begins, it is mostly about pursuing an interesting project, moving to places that we admire, or building a career. Very soon after, a PhD turns into a struggle. If lucky, a couple of successes are sprinkled throughout these years to keep the hopes high. We then survive only through the support of people around us, their kind words, shared problems, strange jokes, and funny one liners. Therefore, it is only fitting that every PhD thesis across the world has an acknowledgement section, and the words "without your support, this would not have been possible" addressed to the humanity that keeps science alive. As the work presented in this thesis is a result of my stay in different labs, I would like to thank everyone.

To my *previlige*, for there are many others who are more deserving, hardworking, and better. Fortunately, I had the time and circumstances that enabled me to write this thesis.

To *Toos Daemen,* my guide in Groningen. *Toos*, my interest in doing interdisciplinary research, the wish to not choose between virology-oncology-immunology, and your expertise at its intersection brought me to your lab. Looking at how the thesis turned out to be, I must say it was a success. Thank you! Beyond that, you always encouraged us towards a rational, objective and goal-oriented approach in doing science. Your wisdom of "do one thing but do it well" will forever stay with me. You have been my first and most critical reviewer, which has very much helped me improve my work, consider my audience, and build my self-awareness. I look forward to continue working with you in the upcoming period; especially in finding my elusive research niche as you found yours with *therapeutic cancer vaccines*.

To *Roger Chammas,* my guide in São Paulo. *Roger*, we first met during my masters internship, and you encouraged me to pursue a double PhD between São Paulo and Groningen. This led to the beginning of this PhD. In the course of my PhD, you have resolved a lot with a short email of "let's make it happen", a feat of magic. You have taught me the simple habit of reading and presenting papers, and broadened my understanding of cancer immunology through our discussions on the *Primer to the Immune response*. I must say that quite a number of things worked out finally because I followed your advice "go for it, it's a good exercise". Thank you for that. On a personal note, my parents and I thank you for helping me organizing my stay in São Paulo during the pandemic. It would have been impossible to continue my PhD far away from home, in a chaotic world, amidst such uncertainties, without all the care I received from you and *Luciana* during that period.

To *Luciana N.S. Andrade*, my daily supervisor in São Paulo. *Lu*, you deserve all the credit for supervising the vesicle experiments, lab organization, all the administrative support, and literally translating everything in English for me. Your

expertise and love for extracellular vesicles has made our collaboration fruitful. Our discussions about scientific life, personal troubles, and the of the pandemic craziness have kept me sound during my stay in Brazil. *Muito obrigado!*

To *César Oyarce Díaz*, my *co-supervisor* in Groningen. *César*, thank you for introducing me to Incucyte, for all the work discussions, and your forever smiling-relaxed attitude. You always encouraged us to be cheerful and positive about life, offering an important perspective amid occasional discouragements in the lab.

A special thanks to *Franjo Weissing*, my guide in theoretical biology. *Franjo*, I remember writing to you during the first year of my PhD, requesting your help for applying game theory to improve oncolytic virotherapy. What followed as a purely theoretical project was the most fun and unique experience I ever had. You welcomed me in although I did not have any background in theoretical biology or computational sciences, invited Thijs to help me with the programming, and patiently allowed the project to develop. Working with you has inspired me to not overlook trivial observations. You provided me with insights and examples on writing a scientific text, and promptly answered any of my seemingly simple questions with a thoughtful and enlightening response. We met on the day you retired, and still work on our collaboration many months later. I think it would be nice to 'retire' the way you did, still working on your projects happily, loving your work, staying curious. Thank you for showing that it was all worth it.

To *Thijs Janzen*, my programming mentor. *Thijs*, thank you for sharing your knowledge on R, on making informative plots, and writing thousands of lines of code for the model. Your light hearted approach has made all the coding work more enjoyable. I am very happy of our collaboration and the resulting chapters present in the thesis (#7 is my favourite) – it would not have been possible without your support.

To *Dr. Jon Laman*, *Dr. Gorben Piljman*, *Dr. Len Seymour*, and *Dr. Bryan Strauss*, my thesis assessment committee members. Thank you for taking the time to read my thesis and providing your feedback. I hope you found it to be a nice and interesting read.

To *Annemarie Boerma and BaukjeNynke Hoogeboom*, my paranymphs and also my go-to technical experts in Groningen. *Annemarie and BaukjeNynke*, thank you for introducing me to the lab, helping me with all the experiments; especially for the hours spent behind a primitive microscope estimating the titer. More importantly, thank you for cheering me up with your friendship and our conversations on anything and everything that crossed our minds. Your presence is irreplaceable. *Annemarie*, you always said that you don't dance, but that's not true anymore. *BaukjeNynke*, when do we start the yoga lessons in the department?

To *Leandro de Lima Coutinho* and *Nathalia L. Santos*, my peers in São Paulo and my paranymphs in Brazil. *Leandro* and *Nati*, thank you for your company, the tapiocas, pastels, bananinhas and cakes, going out for food and movies, sharing

all our troubles, shedding all the tears, and continuing on with our never-ending experiments. Leandro, you've been a great roommate and one of the few people I've interacted with in person, not just through online meetings, during the quarantine months. Nati, thanks for showing me *Osasco*, being my local interpreter at the visa office and the dentist, and introducing me to *Churros*. Both of you made everything more bearable during the pandemic.

To *Martin Beukema* and *Bram ter Ellen*, my office mates in Groningen. *Martin*, thank you for our everyday conversations on the dilemmas of being a researcher, life in academia, struggles with the system and more importantly on listening one's inner voice. It is nice that we have more opportunities to work together and I hope that we don't miss the fun in doing science. *Bram*, thank you for being the juice-detective, the animation wizard, a perfectionist, and the official prank-master of the lab. I am happy that we finished our PhD around the same time as it provided a perfect opportunity to exchange ideas, test cover designs, plan layouts, and inflammatory propositions.

To my group of *Tumor Virology* in Groningen. To, *Ana Milena Vizcaino Castro*. *Milena*, thank you for hosting me during my initial month in Groningen. Without your hospitality, I might not have had the opportunity to meet *Luisa*. Since then, you and Lennard have been a friend and a family to us. We wish you both success and let's meet again for some games and nice dinner. To *Georgia Koutsoumpli, Amrita Singh,* and *Shipeng Chen,* we spent a very short time together, nevertheless it was nice meeting you and I wish you the best for your future.

To my group of *Experimental Cancer Research* in São Paulo. To *Silvina Odete Bustos*, *Luciana Barros*, *Andréia Otake*, *Renata F. Saito*, *Tharcisio Citrangulo Tortelli Jr*, *Tatiane Katsue Furuya*, *Alexis Germán Murillo Carrasco*, *Camila M. Machado*, and many more for helping me in the lab, around the city and providing critical feedback which has improved my work significantly. Special thanks to *Silvina*, for the warm lunch with Greta, and for the mugs! *Luciana Barros*, thank you for your help in setting up the REDCap platform for the systematic analysis, analyzing the data and generating some of the beautiful plots of this thesis. To *Racquel Hessel*, *Taynah I.P. David*, and many others for all the administrative assistance.

To the members of the *Virology and Immunology (VIRIM) research* group in Groningen. Everyone of you knows your science, but also how to party!
To the supervisors *Anke Huckriede*, *Debbie van Baarle*, *Izabela Rodenhuis-Zybert*, and *Jolanda Smit*, thank you for setting an informal, cozy and welcoming atmosphere in the group, your insightful feedback during our meetings, and for always being reachable. *Anke*, a special thanks for the personal advices, sharing your German stories, the concert invitations to your choir during Christmas, and for introducing me to Franjo. *Iza*, thank you for our spontaneous meetings, sparring over research questions and mini-collaborations. *Debbie* and *Jolanda*, special thanks to you both for your continuous and unwavering support. I look forward to continue working with all of you.

To *Jacqueline de Vries-Idema*. *Jacqueline*, I am amazed that you also live in a local (east)India of Groningen. Thank you for tickling my mind over lunch with funny one liners, helping me move, showing me around Groningen, and teaching me "less is more". Let's plan another lunch soon!

To *Marleen van der Laan. Marleen*, thank you for keeping me interested in protein folding and fundamental virology, for sharing a certain silliness in life, personalized whatsapp stickers, organizing lab borrels and outings, and pitching unexpected laughter bombs.

To *Frederique Visser,* for the sweet and nice company, for sharing your unique and sometimes odd work experiences over the years, and for being the subject of my Sinterklass AI poem.

To *Heidi van der Ende-Metselaar*, for the funny interactions with Marleen and Frederique, helping me with the VMT-BSL training, and sharing your stories of Cooper. We still have to do *Elsa*-meets-*Cooper* one day!

To *Lieske Wekema* and *Saskia Meuleman*, great masters students that teamed up with me to navigate through my PhD experiments. I am happy that you both continued what you liked; doing research on a PhD project of your interest. *Saskia*, I had a lot of fun working with you and playing the Disney jukebox. It is good that you could also continue in the *VIRIM* group, and I wish you the best.

To *Nilima* and *Manas,* my Indian colleagues in the lab. Thank you for a constant reminder of home with all the Indian food and snacks that you brought. To colleagues old and new, *Beto*, *Aurora*, *Federica*, *Ellen*, *Yoshita*, *Lilliana*, *Gabriel*a, *Andres*, *Shuran*, *Felipe*, *Marieke*, *Zeyneb, Sofia*, *Laura*, *Harshad*, and *Fakhri*, thank you for the good times. To our lab secretaries, *Hamideh Ahmed*, *Gina Kenter-Uuldriks*, *Jolanda Oldengarm*, and *Joyce Fongers,* for all the administrative guidance and making life easier. *Gina*, we wait for the next ice-cream.

To *Jake Wintermute* and *Ariel Lindner* for introducing me to the world of Synthetic Biology. To the *iGEM teams* over the years, for an informative experience on doing research with limited time and budget, yet overcoming challenges creatively. To mentors and friends, *Sonja Billerbeck*, *Julia Kamenz*, *Patricia Alvarez Sieiro*, *Rianne Prins,* for sharing mentoring tips and advices, and for a memorable experience at the Jamboree. *Rianne*, it was nice to share our PhD experiences, ideas on high throughput screening, the cure for cancer and fungal infections, and finding *Geocaches*. *Sonja*, thank you for sharing the tips and tricks of navigating research life and academia, and in forming new connections. To *Marjolein Crooijmans*, *Jelmer Coenradij*, *Alicia Maciá Valero*, *Maarten Lubbers*, *Enrique Asin-Garcia*, *N. Amy Yewdall*, *Mart Bartelds*, *Sarah D'Adamo*, *Nico J. Claassens*, and *Sonja Billerbeck*, the members of SynBioNL. Thank you for being a friendly group of people and the lively events we organized so far. *Marjolein*, thank you for being a great co-host on #BenchTalks with SynBioNL.

To *Mrugesh Shukla*, for providing the grounds of my scientific initiation, introducing me to the world of experimental Microbiology and Immunology, and for sharing his invincible curiosity and fun driven attitude for Science.

To *Ma*, for she brought me books, taught me to read, and opened my world. To *Papa*, for all the laughter and the way to find happiness within. To *Hansa Ba (હંસા બા)*, for all the stories and songs she wrote for us, for her care and love, and her will to live forever. You are missed. To *Kumud Ba (કુમુદ બા)*, for sending me sweets, love, prayers, complaining that I should study more and not play too much, and for an original plus unforgettable perspective on life in the Netherlands.

To my family in India, who constantly supported me through out the years, whose names and stories would fill another chapter and a book. સમગ્ર ભટ્ટ પરિવાર અને વ્યાસ પરિવારમાં બધા કાકા-કાકી, માસી-માસા, મામા-મામી, ફોઈ-ફુઆ, બા-દાદા, ભાઈ-બહેન, અને બાળકો ને યાદ.

To *Hans Dieter*, *Ulrike*, *Charlotte* and my family in Germany, thank you for the poems, the cakes and the walks, for Minden is not only one of my favorite places in the world but also another home.

To *Sammy*, *Audrey* and *Anouk*, the three lazy yet playful members of our furry feline family, you taught me to enjoy life while we can. To *Elsa*, for being an infinitely curious puppy, and for forcing me to write this thesis during your short afternoon naps.

Finally, to my dearest *Luisa*, the utmost kind and caring person in my life. Thank you for sharing your life with me, and for bringing love, hope and happiness.

About the author

Darshak Bhatt, was born on March 2, 1995, in Dholka, India. He pursued a bachelor's in Microbiology at M.G. Science Institute, Gujarat University, graduating with distinction in 2015. During his undergraduate studies, he worked with Prof. Dr. Mrugesh Shukla, exploring fungal biodiversity and cultivation. Afterwards, he followed an international master's in Immunology and Biotherapies at the Sorbonne University in Paris, France, graduating in 2017. He performed his master's research at the University of São Paulo, Brazil, supervised by Prof. Maristela Martins de Camargo, focusing on the unfolded protein response in B cells of common variable immunodeficiency patients. In 2018, under the supervision of Dr. Edwin (Jake) Wintermute and prof. Dr. Ariel Lindner at center for interdisciplinary research in Paris, he worked on the developing novel star shaped antimicrobial peptides for an iGEM project to address antimicrobial resistance in farm piglets. Next, he obtained the ATTP scholarship in 2019 to realise a double PhD program, supervised by Prof. Dr. Toos Daemen in Groningen and Prof. Dr. Roger Chammas in São Paulo. Simultaneously, he collaborated with Prof. Dr. Franjo Weissing in Groningen to conduct mathematical modeling of spatiotemporal interactions between viruses, cancer cells, and immune responses. He now continues his work in the field of Oncolytic Virotherapy and struggles everyday to maintain a *work-life-dog* balance.

Beyond research, Darshak co-hosts *Bench Talks with SynBioNL*, a podcast on synthetic biology in the Netherlands, and collaborates internationally with iGEM teams to explore synthetic biology's potential in addressing challenges like antibiotic resistance and designing novel biological therapeutics.

Publications related to this thesis

Bhatt DK, Crooijmans ME, Coenradij J, et al.
A SynBio community comes of age: political, academical, industrial, and societal developments in The Netherlands.
Biotechnol Notes. 2022

Bhatt DK, Janzen T, Daemen T, Weissing FJ.
Modelling the spatial dynamics of oncolytic virotherapy in the presence of virusresistant tumour cells.
PLoS Comput Biol. 2022

Bhatt DK, Chammas R, Daemen T.
Resistance Mechanisms Influencing Oncolytic Virotherapy, a Systematic Analysis.
Vaccines. 2021

Bhatt DK, Wekema L, Carvalho Barros LR, Chammas R, Daemen T.
A systematic analysis on the clinical safety and efficacy of onco-virotherapy.
Mol Ther Oncolytics. 2021

Santos NL, Bustos SO, **Bhatt D**, Chammas R, Andrade LNS.
Tumor-Derived Extracellular Vesicles: Modulation of Cellular Functional Dynamics in Tumor Microenvironment and Its Clinical Implications.
Front Cell Dev Biol. 2021

Bhatt D, Meulman SL, Hoogeboom B, Daemen T.
Oncolytic alphavirus replicons induce immune cell activation and recruitment towards human tumor cells and spheroids.
Submitted.

Bhatt D, Boerma A, Odete Bustos S, Chammas R, Daemen T, Andrade LNS, et al, Oncolytic alphavirus-induced extracellular vesicles counteract the immunosuppressive effect of melanoma-derived extracellular vesicles.
Submitted.

Bhatt DK, Janzen T, Daemen T, Weissing FJ. 2023. Modelling the effects of T cell mediated cytotoxicity on oncolytic virotherapy.
To be submitted.

Additional publications

Domingues ACM; **Bhatt D**; Lepique AP.
Resposta imune e evasão (Immune response and evasion). Book chapter in Oncologia - da molécula à clínica. *SÃO PAULO: Editora dos Editores*. 2022

Beal J, Baldwin GS, Farny NG, et al; **iGEM Interlab Study Contributors**.
Comparative analysis of three studies measuring fluorescence from engineered bacterial genetic constructs.
PLoS One. 2021

Bhatt D, Daemen T.
Therapeutic Vaccines and Cancer Immunotherapy.
Vaccines. 2020

Stan RC, **Bhatt DK**, de Camargo MM.
Cellular Adaptation Relies on Regulatory Proteins Having Episodic Memory. *Bioessays*. 2020

Bhatt D, Stan RC, de Camargo MM. Et al.
Chemical chaperones reverse early suppression of regulatory circuits during unfolded protein response in B cells from common variable immunodeficiency patients.
Clin Exp Immunol. 2020

Beal J, Farny NG, Haddock-Angelli T, et al.; **iGEM Interlab Study Contributors**.
Robust estimation of bacterial cell count from optical density.
Commun Biol. 2020

iGEM Paris Bettencourt
Star Cores, Protein scaffolds for star-shaped antimicrobial peptides.
(2018.igem.org/Team:Paris_Bettencourt) 2018

Honors, awards, grants

2022 AIO PhD Award, travel grant from Dutch Tumor Immunology Meeting, KWF-DTIM, The Netherlands

2020-2023 CAPES PhD scholarship, University of São Paulo, São Paulo, Brazil

2019-2023 Abel Tasman Talent Programme PhD scholarship, Graduate School of Medical Sciences, University of Groningen, Groningen, The Netherlands

2019-2022 Research grant (Euros 4k), Foundation De Cock-Hadders, Groningen, The Netherlands

2018 Gold medal, nominated for the Best Composite Part, Paris Bettencourt, iGEM

2018 Research funding (Euros >100k), various sponsors for Paris Bettencourt, iGEM

2017 International master's scholarship, for internship-abroad from Sorbonne university, Paris, France

Scientific outreach

2021-now Board member, SynBioNL – a student-lead organization to advance Synthetic Biology in the Netherlands

2021-now Co-host of podcast: Benchtalks with SynBioNL, discussing current scenario of synthetic biology in the Netherlands

2021-now Blog contribution, Writing 'bite-sized' articles for general audience at Evobites and Oncobites

2023 Organizer, SynBioNL event, Expanding Horizons, converegence of academia and industry

2022 Participant, Public meeting on Society and Synthetic Cells, hosted by Rathenau Institut

2020 Artistic contribution in CellSpace by David Goodsell to visualize 'New ways of living'

Invited talks and posters

Bhatt DK, Meuleman S, Hoogeboom B, Daemen T.
Evaluating immunogenicity of oncolytic Semliki Forest virus in human cancer spheroids.
Talk | KWF-Dutch Tumor Immunology Meeting 2023, The Netherlands

Bhatt DK, Janzen T, Daemen T, Weissing F.
Modelling the spatial dynamics of Oncolytic Virotherapy mediated immune responses.
Talk | Netherlands Society of Gene and Cell Therapy 2023, The Netherlands
Talk | KWF-Dutch Tumor Immunology Meeting 2022, The Netherlands
Online | Dutch Annual Virology Symposium 2023
Poster | Immunotherapy Of Cancer 2022, Germany

Bhatt DK, SynBioNL.
A SynBio community comes of age in the Netherlands.
Talk | at Building Bridges: Connecting Synbio Communities Worldwide at iGEM Grand Jamboree 2022, France

Bhatt D, Bustos S, Daemen T, Chammas R, Andrade LNS.
Tumor-derived extracellular vesicles boost melanoma response to oncolytic therapy.
Poster | International Society of Extracellular Vesicles 2022, France

Bhatt D; Elisa NS, iGEM Paris Bettencourt
StarCores, Protein scaffolds for star-shaped antimicrobial peptides.
Talk | at iGEM Grand Jamboree 2018, USA

Teaching and supervison

2023 Project mentor, for iGEM Groningen team
Title: Bye-o-film: Combating Medical Implant-Related Biofilm Infections

2022 Project mentor, for iGEM Groningen team
Title: Nanobuddy, A Nanotherapeutic Prevention Strategy Against Avian Influenza

2022 Daily Supervisor, for Master Research project and thesis
Title: SFV-based replicons expressing immune activating transgenes increases immune activation markers and immune recruitment to cancer spheroids. Student: Saskia Meuleman

2021 Teaching assistant for Bachelor course on Cancer Cell Biology, University of São Paulo, Brazil

2020 Project mentor, for iGEM IIT Roorkee team
Title: Pyromancer, Design of Novel Antibacterial Protein Complexes for Drug-resistant Infections

2020 Project mentor, for iGEM Paris Bettencourt team
Title: Synderma, is the pursuit of Synthetic Dermatology, where genetically engineered skin microflora could directly secrete biotherapeutics into the skin

2019 Daily Supervisor, for Master Research project and thesis
Title: Design of Immunogenic Semliki Forest Virus-based Replicons for the Treatment of Cancer. Student: Lieske Wekema