Modeling Immune Response to Tumor Growth

Emma Hjörneby [1], Simon Ng [2], and Henrik von Friesendorff [1]

[1] *Lund University, Biomedical Engineering* [2] *University of California, Los Angeles, Bioengineering*

(Dated: October 19, 2020)

Abstract Our purpose is to recreate, evaluate and try to improve an existing model for tumor-immune response. We compared the tumor-immune response by NK cells and CD8+ T cells and investigated how they react to tumor cells transduced with the NKG2D ligand or not. We can see that both the NK cells and the CD8+ T cells respond with different efficiency, and that together they have the best effect on tumor lysis. We could not fully manage to recreate the model presented, at least in part because the article did not present initial values used to implement the model. Additionally, some improvements could be made to the model; these are presented in the Discussion. To further see how the model can be improved and be updated to match where tumor-immune research is today, we altered the model to reflect immunotherapy treatments developed since the original model was produced.

I. Introduction

Our goal with this report is to recreate the model provided in *A Validated Mathematical Model of Cell-Mediated Immune Response to Tumor Growth* (Lisette G. de Phillis, Ami E. Radunskaya, Charles L. Wiseman, 2005) that describes tumor-immune interactions, with a focus on Natural killer cells and CD8+ T cells [1]. The ordinary differential equations provided in Eqs 1,2, and 3 describe the tumor-immune growth, response and interaction rates. The article also provides relevant parameters based on both mouse and human studies reported in other papers [2][3].

Lymphocytes are white blood cells formed in the body's lymphoid tissue, and are either classified as T, B, or Natural killer (NK) cells. NK cells are a part of the body's innate immune system and have cytotoxic effects on tumor cells without previous exposure [4]. They identify cells presenting antigens but also cells with abnormalities on the cell surface, which are present on cancer cells [5].

The CD8+ T cells are a sub population of the T cells, and just as NK cells, they can be cytotoxic to tumor cells, but they need to have prior exposure to tumor cells [6]. The NKG2D ligand on the ligand transduced tumor cells used in the model is an activator for the CD8+ T cells [1]. Their goal was to understand the dynamics of immune-mediated tumor rejection. As a conclusion to their work, they propose that there may be a relationship between a patient positively responding

to immunotherapy treatments and the patients specific response efficacy of the CD8+ T cell.

Their model builds on previous research and contributes to a broader field of tumor modeling research. For example, some other researchers have focused on understanding and improving chemotherapy regimes. Coldman and Goldie created a stochastic model of chemotherapy with one or two drugs. They divided their tumor population into subpopulations to understand resistance in tumor populations [7]. De Phillis and Radunskaya created a competitive differential equation model incorporating a drug treatment, 'normal' body cells, and generic 'immune' cells. They were able to successfully model "Jeff's Phenomenon", an infrequent and unexpected patient fluctuating tumor response to chemotherapy, and develop improved chemotherapy regimes [8][9]. Still, many other researchers have worked to understand and advance the tumor-immune dynamics and the effects of immunotherapy. Kirschner et al investigated adoptive cellular immunotherapy with a system of ODEs including interleukin-2, generic immune 'effector' cells, and tumor cells [10]. Kuznetsov and Knott built a model of differential equations to investigate what causes tumor growth following many years of dormancy. Their models show that a slight decrease in immune efficacy at tumor cell lysis or a mutation of a tumor cell to not be immune responsive can cause a shift from dormancy to growth [11]. Each model considers slightly different aspects of cancerous tumor growth, whether it is chemotherapy, the heterogeneity in tumor cells, variation in immune cells, or effect of some ligand or cytokine.

Immunotherapy is a type of biological therapy, used as cancer treatment that enhance the immune system in fighting the tumor cells [12]. In the article the tumor cells observed are RMA cells, which is mouse leukemia cells. Since the article was published in 2005, and research on tumor therapy has come very far since then, we will, in addition to recreating the model, also try to present solutions to the flaws that we find in the model, and also present improvements that can be made to the model in light of new research.

II. Methods

A. The Model

The model is based on data from earlier publications where studies have been done on both mice and humans. These experiments were also used to set the parameter values in the model. In the equations, the following three populations of cells are observed:

T(t), tumor cells at time t

N(t), natural killer cells at time t

L(t), CD8+ T cells at time t

The equations that form the model are based on the change in the cell populations due to cell growth and death, cell-cell kill, cell recruitment, and cell inactivation. More precisely, the recruitment of the immune cells, D, is based on the Michaelis-Menten form that is commonly used to model enzyme kinetics and now to build mathematical models of the immune system. The Michaelis-Menten form describes, simplified, how the interaction rate is increased with increased substrate, or in this case, cell concentration. The CD8+ T cell concentration however is additionally stimulated by NK cells recruiting when they interact with tumor cells. The tumor cells are not affected by the NK cells and CD8+ T cells in the same way. The death of the tumor cells by the NK cells is linear which means that more tumor cells are being killed when there is a higher populating of NK cells. The same interpretation could not be justified for the tumor specific CD8+ T cells. That is the reason for the new term D shown in Eq (4). The complexity of D is explained in the article as the observed outcome and not outright underlying biological behavior of the CD8+ T cells [1]. The following set of differential equations form the backbone of the model:

$$\frac{dT}{dt} = aT(1 - bT) - cNT - D \tag{1}$$

$$\frac{dN}{dt} = \sigma - fN + \frac{gT^2}{h + T^2} - pNT \tag{2}$$

$$\frac{dL}{dt} = -mL + \frac{jD^2}{k + D^2}L - qLT + rNT \tag{3}$$

where

$$D = d\frac{(L/T)^\lambda}{s + (L/T)^\lambda}T \tag{4}$$

B. Assumptions

To be able to build the model, a few assumptions were made by the original authors. These assumptions are based on known and accepted knowledge of the immune system, but also conclusions made by the authors. These assumptions can be read in full in the article. Here follows a summary of the six assumptions:

1. In absence of an immune response the tumor cells will grow logistically.

2. Both NK cells and CD8+ T cells can kill tumor cells.

3. Both NK cells and CD8+ T cells respond to tumor cells by increasing metabolic activity.

4. NK cells are always present and active in the system.

5. CD8+ T cells are recruited once tumor cells are present.

6. Each NK cell and CD8+ cell will eventually become inactivated after a number of encounters with tumor cells [1].

C. Parameters

The parameter used are estimated from the mouse experiments in [2]. Some of the parameters have been calculated by the authors and some of them comes from additional sources. A full disclosure of how the parameters were estimated can be found in the original article [1]. For understanding of the model we here want to point out that some of the parameters are the same for all of the variations, and some of them change depending on if the RMA cell has been transduced with a ligand or not.

D. T Cell Immunotherapy Project Extension

As an extension of the original author's work, we decided to attempt to use the model for T cell immunotherapy, a relatively new and promising cancer treatment involving the utilization and augmentation of the body's immune system to fight disease. In T cell immunotherapy, T cells are extracted from a patient, manipulated to target antigens present on cancer cells, expanded to large numbers, and reinfused into the patient [13]. Antigen-specific T cells are manipulated with transgenic T cell receptors or chimeric antigen receptors (CAR) [14]. The two necessary changes to the model are to include a large injection of CD8+ cells and to make the CD8+ cells more effective. **This was achieved by adding a CD8+ T cell population increase in place of or in addition to a tumor challenge at Day 10 and by increasing d, also at Day 10, in Eq 4, representing the maximum tumor lysis rate by CD8+ T cells. The increase in d was tuned until the modified CD8+ and unmodified NK cells could suppress the *nn* system without tumor challenge. In reality, only the newly added CD8+ cells at Day 10 should act with an increased d while the already present CD8+ cells should continue to act at the lower d. However, because the increase in d is not based on any physical condition, it can be taken to represent the increase in effective CD8+ lysis rate averaged over the entire population. Additionally, there are many more injected CD8+ cells than endogenous CD8+ cells at Day 10, though the difference is not large**

enough to say that endogenous CD8+ cells are insignificant.

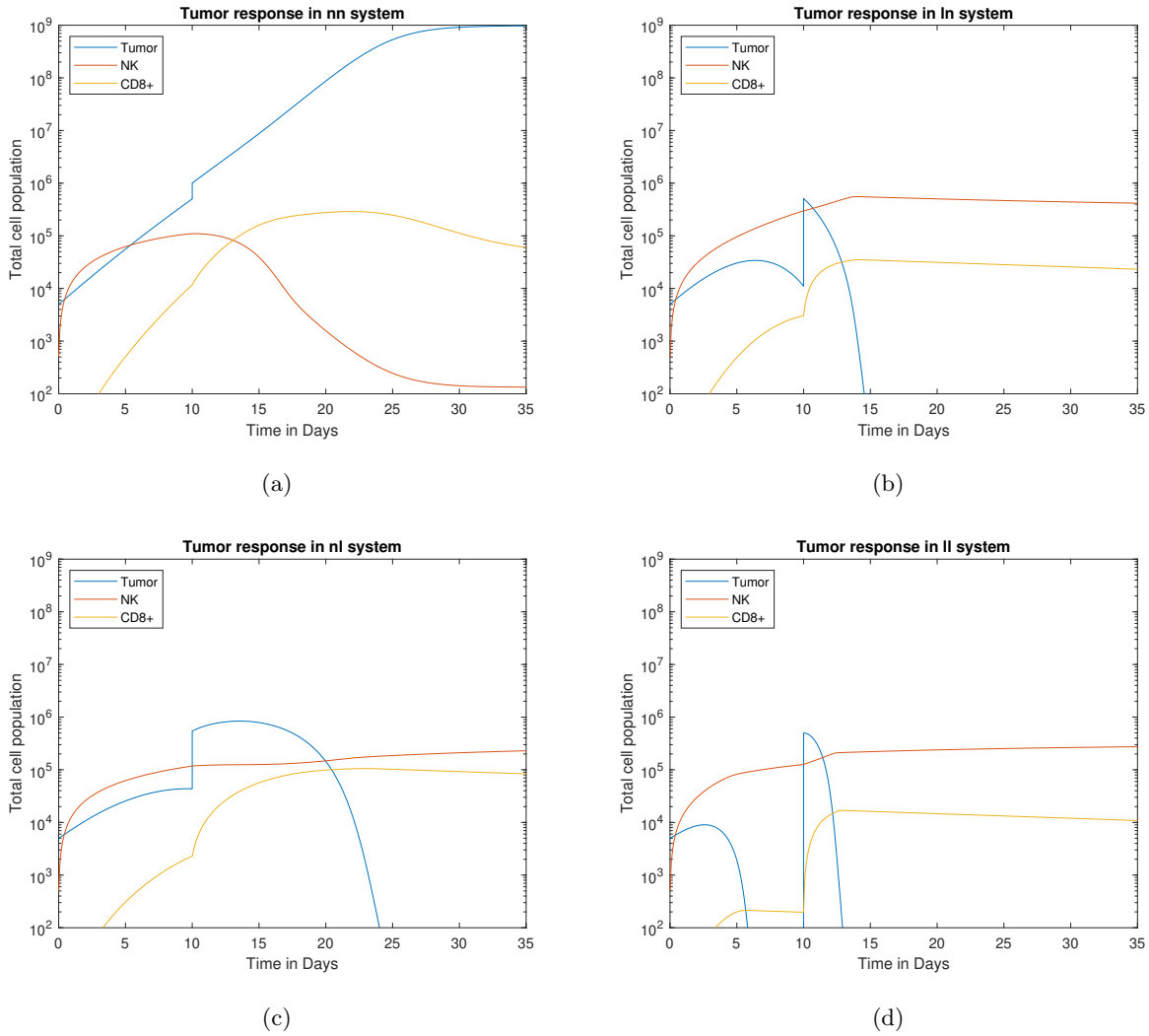


Figure 1: Tumor, NK, and CD8+ T cell population interactions with 5x10^3 tumor cells, 5x10^2 NK cells, and 10 CD8+ T cells. An additional tumor challenge is simulated at day 10 with an addition of 5x10^5 tumor cells. (a) Both initial and challenge tumor populations are control transduced. (b) Initial tumor population is ligand transduced and challenge population is control transduced. (c) Initial tumor population is control transduced and challenge population is ligand transduced. (d) Both initial and challenge tumor populations are ligand transduced.

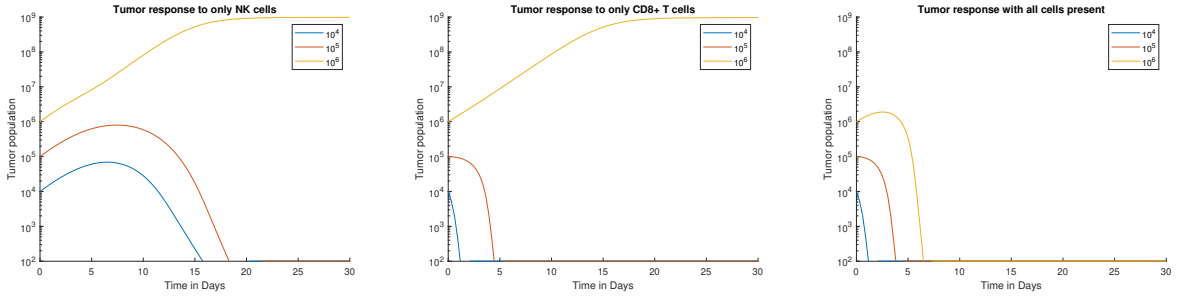
III. Results

A. Tumor Challenge

When none of the tumor cells are ligand tranduced, as in Fig 1a, the NK and CD8+ T cells are unable to suppress the tumor. The simulated dynamics match the equivalent plot produced in [1], giving confidence that the basic model is implemented faithfully. When there are ligand transduced tumor cells in the initial and/or challenge populations as in Figs 1b,c,d, the tumor is suppressed. Interestingly, in Fig 1d when both the initial and challenge tumor cells are ligand transduced, the NK cells seem to begin successfully suppressing the tumor before the CD8+ T cells can even grow significantly. However, prior to challenge on Day 10, it seems that both the *ln* and *ll* systems should behave the same, which they do not. This is addressed further in the Discussion.

B. Suppression Ability

Though [1] did not specify which model parameters were used to produce their equivalent plots to Fig 2, it is logical to use the *ll* parameters since there is only an initial ligand transduced tumor population and no challenge, so the tumor cells are essentially ligand transduced during the entire experiment. Figs 2b,c match the original results, with only the largest tumor population surviving when NK cells are removed in Fig 2b, and with all tested tumor populations being suppressed with both NK and CD8+ T cells present in Fig 2c. However, with CD8+ T cells removed in Fig 2a, the 10^5 tumor population was suppressed in our model, which is not the case in the original results.

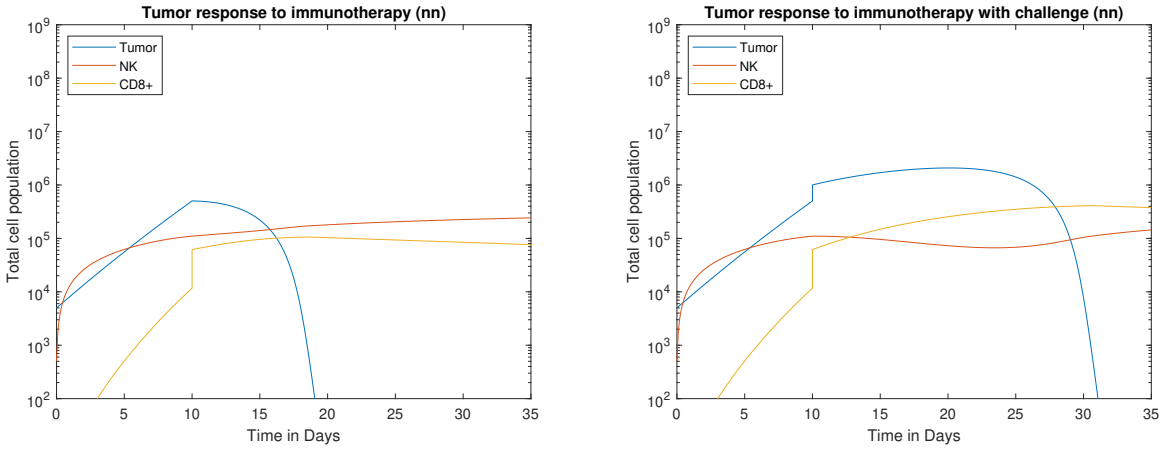


(a) (b) (c)

Figure 2: Tumor growth with different initial challenge population size, 10^4, 10^5, and 10^6 tumor cells, using parameters from *ll* (a) CD8+ T cells depleted (b) NK cells removed (c) both CD8+ and NK cells present.

C. Immunotherapy

Adjusting the model for an immunotherapy treatment produces Fig 3. In Fig 1, the *nn* system was the only one in which the immune system cannot suppress the tumor burden. According to our model, using an immunotherapy such as CAR T cell therapy could provide the necessary immune strength to overcome a tumor burden, even without elevated levels of immune stimulating NKG2D ligand. Even in Fig 3b where the tumor challenge is reintroduced, the enhanced CAR T cells and normal NK cells are able to suppress the tumor. This falls in line with the general consensus that T cell immunotherapy has high potential in cancer treatments. However, we cannot verify our immunotherapy model without experimental data, and so our results are purely theoretical.



(a) (b)

Figure 3: Tumor, NK, and CD8+ T cell population interactions with 5x10^3 control transduced tumor cells, 5x10^2 NK cells, and 10 CD8+ T cells. **To replicate immunotherapy, an additional 5x10^4 CD8+ cells are added at Day 10 with d in Eq 4, the maximum tumor lysis rate by CD8+ cells, increased from 1.43 to 5 for both existing and added CD8+ cells.** (a) No tumor challenge. (b) Tumor challenge of 5x10^5 cells.

IV. Discussion

A. Tumor Challenge

The first plot in Fig 1a looks the most similar to the same plot in the paper (top left in figure 4 of [1]). With parameters from the non-ligand control cells, there are agreeable results both before

and after the Day 10 rechallenge. When the system is both challenged and rechallenged with the ligand transduced tumor cells (the *ll* system) the immunity response is the most powerful, showing the effectiveness of the NKG2D ligand. Even the *ln* and *nl* systems show that systems initially challenged with the ligand transduced tumor cells (the *ln* system) has a quicker response than the system where the ligand cells only rechallange (the *nl* system).

When the initial tumor cells are ligand transduced but the challenge tumor cells are not (the *ln* system), the tumor shows signs of suppression even before the challenge. Fig 1b should match the top right plot in the figure 4 of [1]. However, Fig 1c appears much more closely aligned with the results presented in [1]. In an attempt to make Fig 1b more closely resemble the original results, the initial cell populations were varied widely. However, the overall behavior of the system could not be reasonably modified to resemble the original results.

The authors use one set of parameters for each condition, *nn*, *nl*, *ln*, and *ll* [1]. However, it seems more logical that, before the challenge, parameters should only depend on whether the initial tumor population is control transduced or ligand transduced (n or l), and then parameters should change following tumor challenge to reflect any of the 4 states (*nn*, *nl*, *ln*, or *ll*). For example, the populations in *ln* and *ll* should behave the same before Day 10 when tumor challenge occurs, but because they were given different parameters in this model, they behave differently. This could have been resolved by separately fitting model parameters to the mouse experiments before and after Day 10.

B. Suppression Ability

In Fig 2, we can see the effectiveness of the different immune cells by themselves. The amplified response from the CD8+ T cells relative to the NK cell response can be explained by the tumor cell population. Since the tumor cells are ligand transduced tumor cells and this ligand acts as an activator for the CD8+ T cells, an effective response is expected. Furthermore, the strongest response is in presence of both NK cells and CD8+ T cells.

In Fig 2, we were unable to fully recreate the authors' plots because they did not present how they plotted mean tumor surface area. However, we are confident that the overall behavior of the plots should be the same, with each tumor population size either converging to 0 or growing unchecked. In an attempt to make the system behavior match the authors' results for Fig 2a, we again varied intial concentrations of NK cells, but were unable to achieve the desired behavior. This could be because we had to assume which parameter set was used and chose *ll* because the

original plots used ligand transduced tumor cells. It is possible that the authors used a different parameter set that they did not present in their paper. We did not modify parameters ourselves because it is unclear which parameters to change to reflect their experimental conditions. It is also possible that a different ODE solver could produce slightly different results.

C. Immunotherapy

In 2017 the Federal Drug Administration (FDA) in the US approved two types of immunotherapy using CAR T cells for commercialization [15]. CAR T cells have shown to be successful in eliminating or relieving some types of leukemia. When inserting the CAR gene into T cells they gain an enhanced ability to recognize and attack tumor cells. The assumptions made for the ODEs in [15] are similar to the assumptions made in [1], with the difference that the focus is on CAR T cells and memory cells, and not NK cells. The authors themselves write that their model is not a finished model, but it shows that the dose of CAR T-cells should be adjusted to fit the patient and amount of tumor cells. They produce similar plots as our Fig 3, supporting our modified immunotherapy model. To make an even more predictive model, the models could be combined to include both NK and memory cells.

V. Conclusion

In this report, we have attempted to replicate the results from [1] with some success. We faithfully modeled control transduced tumor growth in the presence of NK and CD8+ cells with a control transduced tumor challenge at day 10. However, we were unable to fully replicate the authors' other results, and the cause for this discrepancy could not be determined from what they published. It would be interesting in future courses to revisit this problem to obtain the raw mouse tumor data from [2] and perform the parameter fitting ourselves with non-linear least squares or some similar method, and then perform cross validation on the results to assess prediction accuracy.

Both NK cells and CD8+ cells are important for the immune response to cancer cells in mice. Together they have the strongest effect. Furthermore is it important to note that there has been a lot of research done on this area since the publication in 2005 and that the model, even though it shows good results, can be updated to reflect the evolving understanding of the field.

[1] L. G. De Pillis, A. E. Radunskaya, and C. L. Wiseman, A validated mathematical model of cell-mediated immune response to tumor growth, Cancer Research **65**, 7950 (2005).

[2] A. Diefenbach, E. R. Jensen, A. M. Jamieson, and D. H. Raulet, Rae1 and H60 ligands of the NKG2D receptor stimulate tumour immunity, Nature **413**, 165 (2001).

[3] M. E. Dudley, J. R. Wunderlich, P. F. Robbins, J. C. Yang, P. Hwu, D. J. Schwartzentruber, S. L. Topalian, R. Sherry, N. P. Restifo, A. M. Hubicki, M. R. Robinson, M. Raffeld, P. Duray, C. A. Seipp, L. Rogers-Freezer, K. E. Morton, S. A. Mavroukakis, D. E. White, and S. A. Rosenberg, Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes, Science **298**, 850 (2002).

[4] Svensk mesh, killer cells, natural, `https://mesh.kib.ki.se/term/D007694/killer-cells-natural` (), accessed on 2019-12-05.

[5] Nationalencyklopedin, nk-celler, `http://www.ne.se/uppslagsverk/encyklopedi` (), accessed on 2019-12-05.

[6] Svensk mesh, cd8-positive t-lymphocytes, `https://mesh.kib.ki.se/term/D018414/cd8-positive-t-lymphocytes`, accessed on 2019-12-05.

[7] A. J. Coldman and J. H. Goldiet, A stochastic model for the origin and treatment of tumors containing drug-resistant cells, Bulletin of Mathematical Biology **48**, 279 (1986).

[8] L. G. De Pillis and A. Radunskaya, A mathematical tumor model with immune resistance and drug therapy: An optimal control approach, Journal of Theoretical Medicine **3**, 79 (2001).

[9] L. G. De Pillis and A. Radunskaya, The dynamics of an optimally controlled tumor model: A case study, Mathematical and Computer Modelling **37**, 1221 (2003).

[10] D. Kirschner and J. C. Panetta, Modeling immunotherapy of the tumor-immune interaction, J. Math. Biol **37**, 235 (1998).

[11] V. A. Kuznetsov and G. D. Knott, Modeling tumor regrowth and immunotherapy, Mathematical and Computer Modelling **33**, 1275 (2001).

[12] Immunotherapy to treat cancer, `https://www.cancer.gov/about-cancer/treatment/types/immunotherapy`, accessed on 2019-12-10.

[13] A. N. Miliotou and L. C. Papadopoulou, CAR T-cell Therapy: A New Era in Cancer Immunotherapy, Current Pharmaceutical Biotechnology **19**, 5 (2018).

[14] M. Aramesh, D. Stoycheva, L. Raaz, and E. Klotzsch, Engineering T-cell activation for immunotherapy by mechanical forces, Current Opinion in Biomedical Engineering **10**, 134 (2019).

[15] B. de Jesus Rodrigues, L. R. Charvalho, and R. C. Almeida, Three-Compartment Model of CAR T-cell Immunotherapy, [] 10.1101/779793 (2019).