# Predicting Long-Term Vaccine Efficacy against Metastases Using Agents

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**Abstract.** To move faster from preclinical studies (experiments in mice) towards clinical phase I trials (experiments in advanced cancer patients), the chance to predict the outcome of longer experiments represents a key step. We use the MetastaSim model to predict the long-term effects of the Triplex vaccine against metastases. To this end we simulate follow-ups of two and three of three months (equivalent approximately to 5.83 and 8.75 years in humans) to compare the long-term efficacy of the best protocol used "in vivo" against the one found by the MetastaSim model. We also check the efficacy of these two protocols by delaying the time of the first administration, in order to catch up the maximum time delay between the appearing of metastases and the administration of the vaccine needed to guarantee reasonable treatment efficacy.

## 1 Introduction

In tumor immunology two main approaches can be identified: Immunoprevention and Immunotherapy. The former attempts to train the immune system in recognizing cancer cells before they appear in the host whereas the latter is based on a series of immunologic treatments administered to cancer patients to eradicate existing tumors.

The Triplex vaccine [4,5] represents a clear example of an immunopreventive approach developed to fight against breast cancer. It combines three different signals for the immune system in the same product. The target antigen is administered in combination with two "adjuvants" represented by IL-12 and allogeneic MHC molecules. The IL-12 adjuvant enhances antigen presentation and Th cell activation whereas the allogeneic MHC molecules stimulate different T cell clones and cause a huge production of various cytokines that amplify immune responses.

This cell vaccine has been capable to totally prevent tumor formation in HER-2/neu transgenic mouse models under a Chronic vaccination schedule in a follow-up time of one year. Shorter heuristic protocols failed in fulfilling this job, leaving unanswered the question of whether a protocol capable of guarantee long term survival with a minimal number of administrations exists. SimTriplex [7],

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an Agent Based Model (ABM) developed to model this "in vivo" experiment, has been used in order to to answer at this question. Combining SimTriplex with well known artificial intelligence optimization methods [15,9,6], led to a schedule with fewer injections which has been now tested "in vivo", yielding to encouraging results [14].

In recent studies [8] the same vaccine demonstrated able (in a follow-up of one month) to elicit a considerable therapeutic activity against metastases derived by mammary carcinoma, thus showing immune responses that overlap only partially those at work in long-term immunoprevention of carcinogenesis. To give biologists the chance to better understand the biological behavior and to predict alternative vaccination schedules, a new ABM named MetastaSim has been developed [12,16]. The model has been used to minimize the scheduling and the number and of vaccinations needed to assure almost complete metastases eradication, predicting a protocol with 40% less injections than the best protocol used "in vivo" (hereafter referred as 1-Triplex).

In order to move faster from preclinical studies (experiments in mice) towards clinical phase I trials (experiments in advanced cancer patients), the chance to predict the outcome of longer experiments represents a key step. In this paper we use the MetastaSim model to predict the long-term effects of the vaccine against metastases. To this end we simulate follow-ups of two and three of three months (equivalent approximately to 5.83 and 8.75 years in humans) to compare the long-term efficacy of the best protocol used "in vivo" against the one found by the MetastaSim model.

Moreover we check the efficacy of these two protocols by delaying the time of the first administration, in order to catch up the maximum time delay between the appearing of metastases and the administration of the vaccine needed to guarantee reasonable treatment efficacy.

The paper is structured as follows: Section 1 introduces the problem. In section 2 we will give a brief introduction of the biological background. In section 3 we will recall the MetastaSim model. In Section 4 we will describe the long-term simulations and we will show the obtained results. Finally in section 5 we will draw final conclusions and considerations.

## 2 Biological Background

Both the immunopreventive and the terapeutic experiments use BALB-neuT female mice models . After birth BALB-neuT mice develop cells hyper-expressing HER-2/neu gene product (p185) in mammary glands. From these cells multiple microscopic lesions arise becoming identifiable as atypical hyperplasia, then progressing to carcinomas in situ, up to macroscopic lesions detectable at around 4-5 months of age.

The therapeutic experiment to test the Triplex efficacy against lung metastases lasts for 32 days. At day 0 all mice receive an intravenous injection of $2.5 \cdot 10^4$ cancer TuBo neu cells (referred to as Neu/H-2), which are used to induce experimental metastases in syngeneic BALB-neuT mice. Then mice are divided in

three different sets: an untreated or control set, a first set which is treated with a protocol (vaccination schedule) composed by a twice-weekly vaccination cycle started one day after the injection of the metastatic cells and repeated up to the end of the experiment (1-Triplex protocol), and a set of mice treated with the same cycle started 7 days after the injection of metastases and repeated up to the end of the experiment (7-Triplex protocol).

We note here that instead of waiting later tumor stages where the breast cancer gives rise to the metastatic burden, experimental metastases are induced artificially in healthy mice because mice in late tumor stages present multiple problems, such as an aged immune system. Moreover surgical remotion of primary tumors cannot be achieved easily, and it is also not possible to exactly establish if and when the metastatic process starts.

The Triplex vaccine stimulates immune system responses using the following stimuli:

- The p185neu antigen, product of the rat HER-2/neu gene;
- H-2q MHC molecules (allogeneic for H-2d BALBneuT mice);
- Interleukin-12 (the cells are engineered with the genes coding for murine IL-12).

The p185neu represents the target antigen recognised and by the immune system responses. The H-2q MHC class I molecules favor recognizing by multiple cytotoxic T cell clones and cause a huge production of various cytokines that amplify immune responses. The IL-12 enhances antigen presentation, helper T cell (TH) activation and secretion of interferon-$\gamma$ (IFN-$\gamma$) by natural killer (NK) and TH cells. IFN-$\gamma$ also has a cytostatic activity on cancer cells (CC) and stimulates granulocytes and macrophages (MP) in infiltrating tumor cell nests in the lungs. Activated TH cells release various cytokines such as interleukin-2 (IL-2) which enhances cytotoxic T cells (TC) activities and releasing of antibodies (Ab) by plasma B cells.

At the end of the experiment (day 32) all mice are killed and lungs are examined to detect the number of formed metastatic lesions. Mice from the untreated set showed $\approx 200$ metastatic nodules; mice treated with 1-Triplex and 7-Triplex protocols respectively showed a reduction $> 99\%$ and $\approx 87\%$ in the number of visible lesions.

## 3 Brief Description of the MetastaSim Dodel

MetastaSim can be defined as "Agent-Based like Model" or as an "extended Cellular Automaton" and uses the same computational framework of the SimTriplex model [7]. An exhaustive description of the model and the modeling framework in general can be found in [16] and in [7], so here we only limit to briefly sketch the model. MetastaSim uses a bi-dimensional 128x128 lattice with hexagonal geometry. The lattice represents a slice of tissue of the frontal ventral surface of mice left lung, and covers a surface of approximately $64mm^2$ and a thickness of $1mm$. Every cell represents an agent with its own life-time, biological behavior,

position in the lattice, set of internal states and one or more receptors. Molecules are represented by their concentration per lattice-site, and by their molecular composition in the case of antigens and antibodies. Relevant immune system entities are modeled and randomly distributed on each lattice-site according to their leukocyte formula. Binary strings are used to represent cell receptors and the molecular structures of antibodies and antigens. The interaction probability of two entities is a function of the Hamming distance between their binary strings. This process mimics well real receptor binding and it s able to reproduce relevant biological phenomena, as shown in [10]. At each time-step ($\Delta t = 8$ hours) all entities that lie in the same lattice-site can probabilistically interact with each-others. Obviously only interactions that are immunologically correct and relevant are allowed. As a consequence of an interaction entities can change their internal status, can release other entities (i.e. plasma B cells release antibodies), can duplicate or can be killed. After the interaction phase ends, entities can probabilistically move to a lattice site in their neighborhood.

One biological assumption that has been made is that every nodule has originated from an individual cancer cell. This means that only one cancer cell over 100 is able to pass through lung capillary vessels and settle into the lungs. This settlement process is not modeled and the simulation starts by supposing that cancer cells have already settled in the lungs. In order to model the same nodule kinetics observed in "in vivo" experiments, it is possible to observe that nodule growths are usually proportional to the quantity of nutrients available. However nutrient distribution in lungs is not known and, due to their extremely high vascularization, neither easily predictable. To reproduce the same nodule distribution in sizes observed "in vivo", sizes and number of nodules data from 8 different real mice are then used with the inverse transform sampling method [2] to generate n random nodule measures that are distributed according to the "in vivo" experiment. These sizes are converted into parameters for the Gompertz growth [1] law and used to compute the duplication probabilities that cancer cells belonging to the same nodule have. After a tuning phase where 8 virtual mice were used to determine the optimal value of free parameters, the model has been validated "in silico" by using 100 virtual mice. As result it showed a good agreement with the "in vivo" experiment [16]. Then its first application has been to search for a protocol capable to assure against lung metastases the same protection entitled with the use of the 1-Triplex protocol.

According to biologists' opinion, no more than two vaccinations per week (in pre-established days) can be done. This needed to satisfy some wet biology requirements (i.e. vaccine preparation) and to guarantee a certain level of safeness for the mice (i.e. avoid undesirable effects or exposition to excessive stresses). The 1-Triplex protocol already uses all the available 9 days to vaccinate. Shorter protocols should be therefore obtained by removing some injections from 1-Triplex protocol. MetastaSim has been then used to explore exhaustively the search space of $2^9$ of possible protocols, finding a five injections protocol (hereafter referred as Optimal) able to give rise to similar protection entitled with the use of 1-Triplex protocol [16]. The protocol is composed by the three injections

in the first 3 available days (days 1,2, and 3), followed by two vaccine recalls (at days 5 and 7). It is worth to note here that all high ranked found protocols share the same structure, i.e. a boost of three injections followed by some (more or less) equally spaced vaccine recalls.

## 4 Long-Term "in silico" Experiments

In previous simulations both the 1-Triplex and the Optimal protocol were able to elicit almost complete eradication (approx. 99% in the number of prevented nodules), whereas the "in silico" 7-Triplex prevention was estimated to be only around 82-83 %. From a translational point of view the time-length of this first experiment is probably too short to investigate long term experiment results. Life span in BALB - NeuT mice is usually around 2 years whereas medium life-span in humans is around 70 years. It is therefore possible to consider a scale factor of around 1 to 35. This means that the first experiment would cover a period of approximately 3 years in humans, whereas the critical the time window for the appearing of recidivous cells is usually considered to be 5 years or more.

The first scenario we simulated is therefore composed by an experiment with a time-length of two months. The 1-Triplex and the Optimal protocol are administered only for the first month. This scenario would allow to understand whether metastases are able to start their growth again after the first month or if 1-Triplex and the Optimal protocols are able entitle prevention for longer times, and if they catch the same prevention. At the same time we also checked the 7-Triplex efficacy on the same scenario to show if the protocol is somewhat able to recover the gap between its entitled efficacy and the other two protocols. We will also checked what happens if we administer the 1-Triplex and the optimal protocol for the first two months in a three-months follow up. All the simulations are executed on a randomly selected 100 virtual mice set, and then the median and the mean number of visible metastases over the entire set are taken as outcome. Most important mean entities behaviors are shown as well. Protocols efficacy is measured considering the total number of entitled metastatic nodules a the end of the experiment. Best protocols will have lower medians and means. 1-Triplex and 7-Triplex protocols are extended by repeating the twice-weekly cycle for the required periods. The 2 and 3 months extensions of the Optimal protocol are obtained by repeating the injection schedule of the first month one or two more times respectively.

We remark here that only visible nodules (i.e. nodules that have reached a minimum number of cells and should be visible in "in vivo" lung examinations) are considered. In some cases, even if there is no evidence of visible metastatic burden (such as visible nodules), cancer cells may be still present in a small number but taken under control by the immune system. Moreover tumor multiplicity (presented in tables 1 and 2) is not strictly connected with the total mean number of cancer cells (shown, for example, in figure 2) present in the system. The former represents a measure that can be checked and compared with the "in vivo" results, the latter is only presented to deeply investigate and analyze

the system and does not have an equivalent in the "in vivo" experiments. Table 1 reassumes the results coming from the previously described scenarios. The first three rows refer to the one-month experiments already published in [16] and are reported for comparison. In a two months follow-up it's easy to see that the median and mean numbers of metastases entitled with the use of Triplex-1, Triplex-7 and Optimal protocols remain substantially unchanged.

The use of the 1-Triplex and Optimal protocols for two months in a three months follow-up indeed shows how both the two protocols mostly eradicate the metastatic burden. In figure 1 the behavior of some of the involved cells and molecules is showed for this last scenario. Even if the immune response of B and T helper cells is slightly weaker in the Optimal protocol, the mean cancer cells curves of the two protocols are practically indistinguishable. This confirms the redundancy of injections in the 1-Triplex protocol. The lack of some spikes in the Ag graph for the Optimal protocol is justified by the lack of some injections in respect to 1-Triplex protocol. We point out here that these spikes are due to the fact that every vaccine injection introduces new vaccine cells that are easily killed and release antigens as result. Antigens are then rapidly captured by antigen presenting cells and presented to B and T cells to stimulate the immune response. The difference in efficacy between the first two protocols and the 7-Triplex suggests that the latter is probably started too late to entitle total efficacy. The knowledge of the maximum time delay between the injection of metastases and the administration of the vaccine needed to guarantee metastases prevention thus represents a question that should be answered. To this end we simulated a follow-up of three months where the 1-Triplex and Optimal protocols are administered by delaying the start of the treatment of 3, 5, and 7 days. From table 2 it is possible to note that even in this case both protocols allow similar protection rates. A 3 days delay in the start of the treatment does not particularly affect the efficacy of the protocol. Starting from a delay of 5 days it is possible to observe a worsening of the final outcome of the experiment.

To better investigate this scenario it is possible to look at figure 2 where the mean behavior of the total number of cancer cells for the entire time-length of the experiment is displayed. Both the protocols show negligible differences in general and are able to destroy all cancer cells in no more than 65 days when administered with no delay. A delay of 3 days postpones the metastatic cells elimination of approximately 10 days. If a 5-days delay is taken into account, both the treatments are not able to completely eliminate all cancer cells in 3 months even if administered for the entire period. However it is possible to observe that cancer cells curves are decrescent for the 5-days delayed protocol, suggesting a possible complete depletion if longer times are taken under observation. As previously suggested, a protocol delayed by 7 days seems to be not able to contrast the growth of some metastatic nodules. Even if it is possible to observe a decreasing trajectory between days 15 - 30 due to the fact that nodules with low growing rates succumb to immune system responses, nodules with higher growing rates are already too big to be eliminated by the immune system.

## 5 Conclusions

Cancer represents nowadays one of the most appalling diseases. In particular, metastases represent one of the major concerns in the clinical management of cancer. The majority of cancer mortality is in fact associated with this disseminated disease rather than the primary tumor [3]. Moreover standard protocols for the treatment of cancer usually establish that the risk of recidivous cells last for periods even longer than 5 years. In this optic the use of treatments that can be safely used for long times with minimum risk of collateral effects to substitute, or integrate existing chemotherapy-based treatments can represent a key step in the fight against cancer. However treatment minimization is always advisable. The experimental induction of metastases in tumor-free mice can represent well a typical scenario in human cancer, i.e., the scenario arising after the surgical removal of the primary tumor. Computational modeling of this setup allowed prediction of an Optimal protocol an the "in silico" study of long-term efficacy of the Triplex vaccine.

From results we found that the both 1-Triplex and the Optimal protocols are able to yield to a mostly complete eradication of the metastatic burden if vaccine administrations are continued for two months. From a translational point of view this would suggest that the use of this vaccine in human should be extended to a period of 5 years after surgical intervention. Another major finding comes out from the analysis of the maximum delay needed to avoid the appearing of metastases. MetastaSim suggested a maximum delay of 3 days to achieve complete eradication in a period of three months. This would translate in a maximum delay of slightly more than 3 months in humans. A delay 5 days (which translate to almost 6 months in humans) remains treatable but probably requires longer treatment times. Higher delays may entitle high risk of metastatic occurrence and therefore should be avoided.

We would like to highlight that even if these "in silico" predictions are strictly related to the use of the Triplex vaccine and to the experimental setup it has been tested, such modeling approaches can be applied and integrated to successfully study other diseases and pathologies, such as the ImmunoGrid framework [13,11].

## Figures

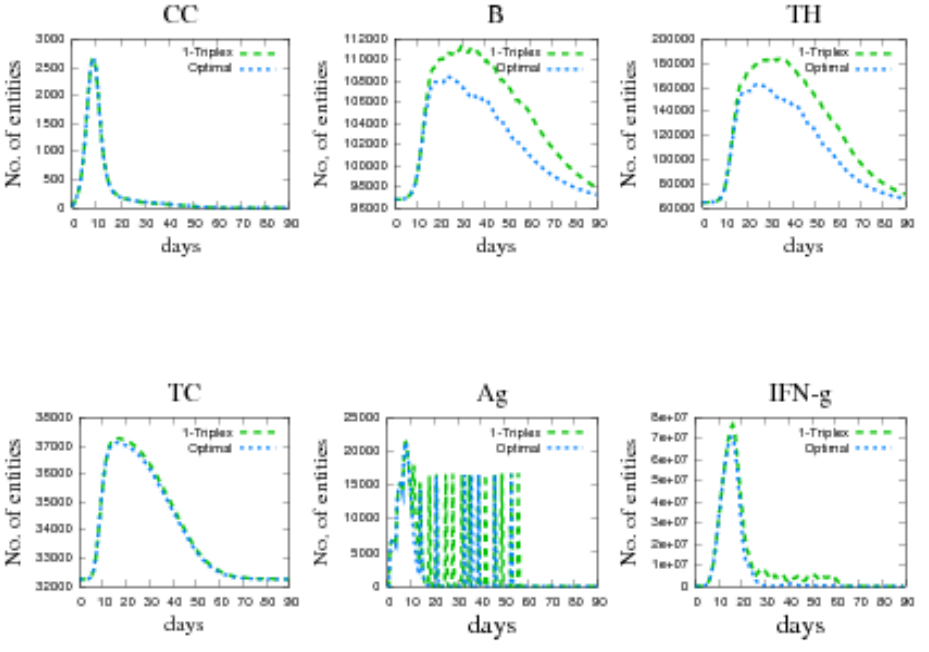


**Fig. 1.** Cancer Cells (CC), B cells, T helper cells (TH), cytotoxic T cells (TC), Antigens (AG),Interferon-$\gamma$ (IFN-g) behaviors with 1-Triplex and the Optimal protocols. The time-length of the experiment is 3 months. The two protocols are repeated only the first two months.

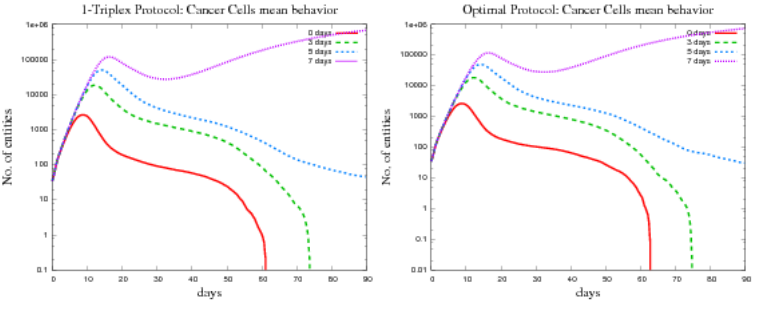


**Fig. 2.** Cancer Cells (CC), B cells, T helper cells (TH), cytotoxic T cells (TC), Antigens (AG), Interferon-$\gamma$ (IFN-g) behaviors with 1-Triplex and the Optimal protocols. The time-length of the experiment is 3 months. The two protocols are repeated only the first two months.

## Tables

**Table 1.** 1-Triplex, 7-Triplex and Optimal vaccination protocols predicted efficacy (entitled number of visible metastases at the end of the experiment). The column "Range" reports the minimum and the maximum number of visible metastases observed over the 100-mice random set on which the protocols have been tested. The columns "Median" and "Mean" show the median and the mean number of visible metastases, respectively.

| Protocol | Experiment length (Months) | Months of Administration | Range | Median | Mean |
|---|---|---|---|---|---|
| 1-Triplex | 1 | 1 | 0 - 2 | 0 | 0.33 |
| 7-triplex | 1 | 1 | 0 - 12 | 5 | 5.57 |
| Optimal | 1 | 1 | 0 - 2 | 0 | 0.36 |
| 1-Triplex | 2 | 1 | 0 - 2 | 0 | 0.35 |
| 7-Triplex | 2 | 1 | 0 - 10 | 0 | 5.39 |
| Optimal | 2 | 1 | 0 - 2 | 0 | 0.33 |
| 1-Triplex | 3 | 1 - 2 | 0 | 0 | 0 |
| Optimal | 3 | 1 - 2 | 0 | 0 | 0 |

**Table 2.** 1-Triplex and Optimal protocols "in silico" efficacy (entitled number of visible metastases at the end of the experiment) with a delay of 3, 5 and 7 days in the time of first injection. The experiment lasts for 3 months. The column "Range" reports the minimum and the maximum number of visible metastases observed over the 100-mice random set on which the protocols have been tested. The columns "Median" and "Mean" show the median and the mean number of visible metastases, respectively.

| Protocol | Delay of the first injection (days) | Range | Median | Mean |
|---|---|---|---|---|
| 1-Triplex | 0 | 0 - 0 | 0 | 0 |
| | 3 | 0 - 0 | 0 | 0 |
| | 5 | 0 - 1 | 0 | 0.02 |
| | 7 | 0 - 2 | 0 | 0.39 |
| Optimal | 0 | 0 | 0 | |
| | 3 | 0 - 0 | 0 | 0 |
| | 5 | 0 - 1 | 0 | 0.02 |
| | 7 | 0 - 4 | 0 | 0.46 |

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