Improving T-cell immunotherapy for melanoma through a mathematically motivated strategy: efficacy in numbers?

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Abstract

T-cell mediated immunotherapy for malignant diseases has become an effective treatment option especially in malignant melanoma. Recent advances have enabled the transfer of high T-cell numbers with high functionality. However, with more T-cells becoming technically available for transfer, questions about dose, treatment schedule, and safety become most relevant. Mathematical oncology can simulate tumor characteristics in silico and predict the tumor response to novel therapeutics. Using similar methods to classical pharmacokinetics/pharmacodynamics type models, mathematical oncology translates the findings into a multi-parameter model system and simulates T-cell therapy for malignant diseases. The tumor and immune system dynamics model, can provide minimal requirements (in terms of T-cell dose and T-cell functionality) depending on the tumor characteristics (growth rate, residual tumor size) for a clinical study, as well as help select the best treatment schedule (repetitive doses, minimally required duration, etc.). Here we present a new mathematical model developed for modelling cellular immunotherapy for melanoma. Computer simulations based on the new model offer an explanation for the observed finding from clinical trials that the patients with the smallest tumor load respond better. We simulate different parameters critical for improvement of cellular therapy for patients with high tumor load of fast growing tumors. We show that tumor growth rate and tumor load are crucial in predicting the outcome of T-cell therapy. Rather than intuitively extrapolating from experimental data, we demonstrate how mathematical oncology can assist in rational planning of clinical trials.

Introduction

Adoptive immunotherapy using tailored T-cell infusions to treat malignancies has been proven to be effective in certain types of malignancy [1,2]. However, our understanding as to why certain patients respond whereas others progress, is still limited. Moreover, clinical approaches using T-cell therapy still vary widely in issues such as how to generate large numbers of specific T-cells, how many T-cells to use for therapy and what schedule would be most effective. The expansion of tumor-specific T-cells has been hindered by the often low precursor frequency in patients or healthy individuals, the loss of high affinity T-cells during ex vivo culture and the terminal differentiation of extensively cultured and expanded T-cells, resulting in loss

of function and persistence upon transfer to the patient [3]. Recently, progress has been made with genetic engineering of chimeric antigen receptors, T-cell receptor transfer and silencing of genes exerting unfavourable functions [4,5]. Furthermore, use of new cytokine combinations allows the rapid expansion of less differentiated T-cells with an enhanced functional capacity [6]. However, recent preclinical and clinical data also reveal the dangers of this type of therapy with several serious adverse events related to T-cell infusions being reported [7-9]. Therefore, with high numbers of antigen-specific T-cells becoming available for clinical use and at the same time considering the potential risks, we asked, whether rather than intuitively deciding on a given T-cell regimen, mathematical modelling would help to define the prerequisites of an effective immunotherapy approach.

Integrative mathematical oncology is widely used to decode the cancerous process [10]. Several mechanistic or descriptive models have been proposed to better understand the growth dynamics of cancerous cells embedded in the non-malignant environment. Part of the models have studied three-dimensional growth of tumors, while others have preferred the use of non-spatial mathematical models [11,12].

In this context modelling tumor-immune interactions has been key issue over the past two decades. De Boer et al. were one of the first to model the interaction between T-cells, macrophages and tumor cells, taking into account a variable immunogenicity of the tumor cells [13]. In this in silico model small variations in specific T-cell subsets, especially the number of activated helper T-cells, had a large impact on tumor cell growth, whereas when computing the required number of cytotoxic T-cell to achieve the same effect was 1000-fold higher. However, Takayanagi and Ohuchi provided mathematical analysis showing that an increased numbers of cytotoxic T-cells may ultimately tilt the balance between tumor and immune system in favor of the latter [14]. Other groups have investigated different aspects of immunotherapy such as the role of tumor dormancy, the therapeutic use of Interleukin-2 (IL-2) and the effects of tumor-associated cytokines such as transforming growth factor beta (TGFß) or epidermal growth factor (EGF) [15-19]. For a comprehensive review of simulation methods of tumor immunology see Woelke et al., 2010 [20].

We recently developed a mathematical model of T-cell therapy for glioblastoma, that includes the mutual interactions of the immunosuppressive role of the tumor and the manner by which major histocompatibility complex (MHC)

receptors expression can be induced by cytotoxic T-lymphocyte (CTL) secreted cytokines [21]. The mathematical model furthermore predicts the existence of a threshold value of T-cell infusion rate, which needs to be reached, before a significant impact on the tumor growth can be observed [22].

In this paper we present a mathematical model for immunotherapy in the context of published clinical data. We chose immunotherapy by transfer of ex vivo expanded tumor-specific T-cells for melanoma patients, as this therapeutic strategy has been shown to be especially effective in this patient population. At the same time little is known about the actual numbers of T-cells required for therapeutic treatment, resulting in a wide variation in T-cell numbers across clinical studies. In fact T-cell dosing is often calculated based on body surface area, - a concept which stems from pharmacological substances which rely on renal excretion – or on body weight, rather than on the estimated tumor burden. However this is exactly where mathematical modeling may help to design an individualized treatment schedule for each patient. Based on reported parameters in the literature, a mathematical model was built by transferring a descriptive model into mathematical equations. Using experimental quantitative data reported recently, the model was then retrospectively validated. We then analyzed treatment schedules used previously in clinical trials, to assess the magnitude of a functional T-cells response required to efficiently target malignant melanoma. We show, that even high T-cell numbers used for therapy will unlikely influence large and fast growing tumors, unless the functional capacity of each T-cell-product is improved. Furthermore, finer quantification of residual tumor load may assist in identifying patients, which will benefit from this type of therapy.

Methodology

In our previous work [21] a simplified mechanism for alloreactive cellular therapy for glioblastoma was introduced. Partially based on this previous work, we developed a new model with special adaptation to melanoma and melanoma immunotherapy parameters. Figure 1 shows a scheme of the model. The mathematical equations are:

Mathematical equations

$$\dot{T} = rT \cdot (1 - \frac{T}{K}) - a_T \frac{M_I}{e_T + M_I} (\frac{a_{T,\beta} \cdot F_\beta + e_{T,\beta}}{F_\beta + e_{T,\beta}}) \cdot C \cdot T \frac{h_T}{h_T + T} \qquad (1)$$

Eq. (1) describes the tumor cell, *T*, dynamics, (the dot is the time derivative), as influenced by tumor cell maximal number, K, CTL population, *C*, killing efficacy of the CTLs, a_T, presence of MHC class I receptors, M_I, and the level of TGFβ secretion. For full explanations here and hereafter and list of parameters see Appendix.

$$\dot{C} = g_C - \mu_C \cdot C + I \qquad (2)$$

Eq. (2) describes the dynamics of the CTLs, *C*, determined by the inflow rate of CTLs, g_C, and the death rate of CTLs proportional to the population of CTLs, with coefficient μ_C , and finally *I*, the CTL infusion rate.

$$\dot{F}_\beta = a_{\beta,T} \cdot T - \mu_\beta F_\beta \qquad (3)$$

Eq. (3) describes the dynamics of TGFβ, F_β, as proportional to the tumor cell population, *T*, with $a_{\beta,T}$ as a proportion coefficient and is destroyed at a rate of μ_β proportional to F_β.

$$\dot{F}_\gamma = a_{\gamma,C} \cdot C - \mu_\gamma \cdot F_\gamma \qquad (4)$$

Eq. (4) describes the dynamics of IFNγ, F_γ, as proportional to the population of CTLs, *C*, with proportion coefficient, $a_{\gamma,c}$, and is destroyed at a rate μ_γ proportional to the amount of F_γ.

$$\dot{M}_I = g_{MI} + \frac{a_{MI,\gamma} \cdot F_\gamma}{F_\gamma + e_{MI,\gamma}} - \mu_{MI} \cdot M_I \qquad (5)$$

Eq. (5) describes the dynamics of MHC class I molecules, M_I, are presented on the tumor cell cell surface at a rate g_{MI}, this rate is augmented by a Michaelis type term dependent on F_γ**.** The M_I molecules are internalized at a rate μ_γ.

Model assumptions

The current model assumes that a primary tumor cell population and a metastatic tumor cell population are indistinguishable by size alone or level of malignancy per metastatic population. We feel it is a safe assumption to make for most of the patients, at least at this fundamental stage of model development.

Computer simulations

Computer simulations were performed using Matlab. For all simulations, tumor initial population size was determined between $1x10^{10}$ cells to $5x10^{11}$ cells. We divided this range into 50 equal intervals. Tumor growth rates were set between 0.0001 h^{-1} to 0.001 h^{-1}. In the same manner that range was divided into 10 equal intervals. All combinatorial possibilities were then crossed, creating 50x10=500 combinations of tumor initial population size and tumor growth rates. The crossing imitates a virtual population of patients with a wide range of personal disease characteristics. Computer simulations predicting tumor population size under different adoptive T-cell therapy regimens over 300 days were run with these initial conditions. Out of this 500 virtual patients' grid (or data set), 100 patients were selected randomly and their simulation results were presented in plots and summary statistics. The results of randomly selected 100 patients were plotted.

Clinical interpretation of computer simulation results

To convey the clinical relevance of the simulation results we used the RECIST criteria for imaging response. We therefore demarcated simulated tumors in the figures according to their RECIST score. Tumors that have reduced in size to practical zero at the end of the simulated time span were considered RECIST complete response, CR; tumors that have reduced by at least 30% were considered RECIST partial response, PR; tumors that have stabilized between 70% and 120% of their original size were considered RECIST stable disease, SD; tumors that have grown beyond 120% of their original size at the end of the simulated time span were considered non-responsive, NR.

Results

Description of the model

This mathematical model simulates the effect of cytotoxic lymphocytes on melanoma cells (Fig.1). It consists of 5 differential equations describing the dynamics of the tumor growth, CTL persistence, cytokine secretion and MHC-class-I-expression. The exact dynamics is controlled by the following key parameters: cytolytic activity of the T-cells, tumor growth rate, survival of the T-cells and the collective sensitivity of the tumor cells to killing (h_T). It is a refined model of our previous work modelling immunotherapy for glioblastoma patients [22,21]. A detailed description of the model and all the relevant parameters is given in the Appendix.

Selection of studies simulated with this model

We wanted to simulate four representative immunotherapy studies based on the data obtained from published literature in order to model the greatly varying treatment schedules and results [23-25]. The four studies were selected using the following criteria: clinical trials (phase I/II) using antigen-specific T-cells to target malignant melanoma; data available in the literature to model treatment schedule in terms of dose and timing, different research groups with only one study per group.

The four selected studies are summarized in Table 1 illustrating the significant variation in terms of T-cell numbers administered, frequency of infusions and initial tumor load. Therefore, these main factors were analyzed with this model.

Polyclonal T-cell lines in stage III melanoma

Khammari et al. compared infusions of ex vivo expanded tumor infiltrating lymphocytes (TIL) with IL-2 versus IL-2 treatment alone [23]. Eighty-eight patients were enrolled and equally distributed into each arm of the study. These patients had not received prior systemic treatment and were enrolled when regional lymph node recurrence occurred after surgery (Stage III, T1-4N recurrent M0). The group reports on a long follow-up of more than 10 years.

Patients with one invaded lymph node were compared to patients with multiple lymph node metastasis. Patients with only one invaded lymph node receiving TIL and IL-2 had a striking survival advantage over patients receiving IL-2 only (33.3% vs. 68.42% relapse percentage). This difference was not seen in patients with multiple lymph node metastases, suggesting that tumor load impacts efficacy of the treatment. Tumor load and tumor growth rate for each individual patient, however, are not reported in the study. [21,22]. To model this study, we therefore chose a range of initial tumor sizes and tumor growth rates (see Methodology section for details) and simulated the potential outcome for 500 different combinations of initial tumor load and growth rate values (Figure 2). In analogy to the clinical protocol, we simulated a treatment regimen with two infusions given one month apart. Simulations were performed using the reported median cell dose of 1.7×10^{10} TIL (Fig. 2A), as well as the lowest (2.2×10^{9}, Fig 2B) and highest (2.7×10^{10}, Fig 2C) doses. As the best reported value of tumor specific T-cells was 13.8%, T-cell dose was corrected for 10%.

Simulated response rate varied between 1% and 25% (Fig. 2B and 2C) depending mainly on TIL dose. Since one assumption of this model is the capability of T-cells to serially lyse tumor cells, a correlation between the number of T-cells infused and tumor growth inhibition was to be expected. However, even with the highest T-cell dose, and assuming favorable conditions for the T-cells, only tumors with the lowest size could be delayed significantly in their growth or reduced in size reflecting complete remission according to RECIST criteria. Thus the mathematical model predicts that even when assuming repetitive lysis of tumor cells by each T-cell infused, only in rare circumstances, such as low residual tumor size, a significant impact is to be expected.

The fact that only patients with involvement of a single lymph node showed a benefit in this study, suggested a quantitative effect of the T-cells in the tumor load. However in a subgroup analysis of the 27 patients for whom data on the content of tumor-reactive T-cells were available, no correlation was found between clinical outcome (as indicated by relapse vs. no relapse) and the applied dose of antigen-specific T-cells [26]. One likely explanation may be the small sample size. Computer simulation offers an alternative explanation: Figure 3 plots initial tumor size versus simulated final tumor size. Simulations are shown for the same 3 different T-cell doses. For tumors larger than $1x10^{11}$ no correlation can be seen between outcome (final tumor size) and initial tumor voulume, regardless of the T-cell dose infused. This is in line with the clinical observation. Only when the initial tumor burden is sufficiently small, a dose-effect relationship of the T-cells can be established, suggesting that rather than clinical staging as was done in this trial, a detailed volumetric analysis of residual tumor masses – if at all possible – may represent better correlation with clinical effectiveness of a T-cell response. TIL dose-effect relationship can be seen only for small initial tumor size and completely vanishes for large initial tumor cell numbers (Fig. 3).

T-cell clones with defined specificity

The exact fraction of antigen-specific T-cells within the infused T-cell lines remained unknown in the study by Khammari et al. To address better the actual number of antigen-specific T-cells, we simulated a different study, in which clonally expanded antigen-specific T-cells, thus suggesting similar tumor-reactivity, were used [25]. Ten stage IV melanoma patients (metastatic disease) were treated with $3.3x10^{9}/m^{2}$ CTLs

given in bi-weekly infusions 4 times – with 2 patients receiving 6 and 7 infusions respectively, and 2 patients receiving only 3 infusions. Detailed data on initial tumor burden are not available. Yee's clinical data report on 7 out of 10 patients as having partial responses, 5 of which reaching stable disease with a mean duration of about 1 year and 2 patients with progressive disease. Mathematical modelling suggests a 59% response rate (complete and partial responses) at 300 days post-treatment (Fig.4). Bearing in mind that the actual tumor burden or tumor growth rates of the patients were not available, the agreement between the clinical trial and the simulations is good. The number of antigen-specific T-cell numbers of each infusion in Yee's trial was comparable to the highest T-cell dose infused in the Khammari study, however 4 infusions were given in Yee's study as opposed to 2 infusions in the Khammari trial. This may account for the higher response rates – observed clinically and simulated mathematically – in Yee's trial.

Multiple infusions with short-term cultured T-cell lines

To address the question, how the number of infusions affects treatment success, we chose a study performed by Mackensen et al. Polyclonal T-cell lines were generated against a single epitope (MelanA/MART1) and infused (average: $2.1x10^8$ T cells). Three T-cell infusions were scheduled two weeks apart, followed by up to 10 infusions in 4-week intervals. IL2 was administered for 6 days after each infusion. Eleven patients were treated [24]. One patient achieved a complete response and one patient achieved partial response; two others had stable disease and mixed reactions. Computer simulations of randomly selected virtual patients showed a positive response rate of 10% (at day 300 post-treatment), which is lower than the reported clinical data (36%, 3 months minimal observation time) (Fig 5A). Next, we used the model to search for a more effective regimens which were not observed in Mackensen's clinical trial. Figure 5B shows that all other parameters being equal, increasing the T-cell dose to $5x10^8$ cells (which is still 10 times less than the dose apllied in Yee et al.) increases response rates within the simulation to 20%. When T-cell mediated tumor lysis parameter was increased 2-fold (see Table 1 in Appendix) a positive effect was observed on 24% of the simulated patients (Fig 5C). Combining higher lytic capacity and increased T-cell dose lead to a greatly increased efficacy of 34% (Fig 5D).

Excessive T-cell numbers combined with chemotherapy

Whereas experimentally it is possible but difficult to assess differences in lytic capacity between different clinical studies, due to differences in handling the T-cells and performing the assays, T-cell numbers are more comparable. A correlation between cell numbers and clinical efficacy was already established with the aforementioned simulations. However, the magnitude of the T-cell dose needed to achieve durable responses in most of the patients was unclear. We therefore analyzed data of a clinical trial performed by Steve Rosenberg's group, in which a very high number of T-cells was used, – in fact, to the best of our knowledge, it is the highest number of T-cells used and reported for adoptive T-cell therapy [27,28]. These studies have used non-myeloablative chemotherapy prior to the TIL-infusion. A total of $1x10^{11}$ cells were given in one bolus infusion, and more importantly total body irradiation (TBI) therapy in a low dosage (2 Gy) and in high dosage (12 Gy) was added to the therapy [28]. The positive response rate was 13/25 (52%) and 18/25 (72%) respectively. In the simulations we show here, we chose to simulate the high dose treatment assuming antigen specificity of 10% based on Table 3 in Dudley et al. 2008 [28]. When this high dose was given alone, a positive response in 52% of the patients was predicted (Fig.6A). Next, we simulated a possible effect of low intensity TBI immediately prior to the TIL infusion. We assumed that such a treatment could decrease tumor load. Figure 6B shows that reducing tumor load by 10% increased positive responses to 56%. To simulate the high TBI treatment, we assumed that in addition to tumor size reduction the high dose TBI sensitizes tumor cells to CTL mediated lysis (e.g. by an increase in tumor accessibility due to pro-inflammatory signals). Such a change in the tumor-CTL accessibility is reflected in the parameter, h_T. A simulated increase of 33%, results in a similar positive response in 71% of the virtual patients (Fig. 6C). The accessibility parameter h_T is used in modelling tumor-immune interactions to attenuate the killing efficacy of CTLs as the tumor increases (for instance de Pilllis et al. [29]). Of all parameters used, it is the most difficult parameter to assess experimentally, as multiple events leading to a potential increase in sensitivity of the tumor cells for T-cell mediated lysis may be represented by this parameter (e.g. the elimination of regulatory T-cells). However it is a valid alternative explantion to increased efficiency of T-cell immunotherapy through a general effect on tumor sensitivity due to the preparative chemo-/radiotherapy, which results in higher response rates.

Discussion

Our mathematical model, simulating four independent clinical trials, emphasizes three critical issues for this type of therapy: 1) cellular therapy is based on the functional capacity of each individually transferred cell, 2) even high T-cell numbers used for therapy will unlikely influence large and fast growing tumors, and 3) the current classification of tumors (stage and grade) needs to be refined for proper estimation of the residual tumor cell mass. Future model modifications may include classification of tumors by the metastatic sites and their malignancy scores. Currently we feel however that even population size alone can provide us with ample insights and predictions as to the success of T-cell therapy.

Absolute tumor burden and growth dynamics can be estimated within a certain degree of certainty at the start of cellular therapy taking into account size, stage and grade of the tumor. As demonstrated here, low initial tumor cell mass is critical for the success of T-cell therapy. For instance, although Mackensen et al. [24] do not report exact tumor burden it is hard to ignore the fact that all patients that showed any response at all (mixed, partial, or stable disease) were the ones with only *one* disease site (Table 1 in Mackensen et al. 2006), which is consistent with Khammari's findings. This underlines the importance of quantitative effects in cancer progression and the existence of a window of opportunities for efficacious treatment.

In the simulations presented here, both tumor cell numbers and growth rates were randomly chosen within a pre-set range to simulate various biological preconditions. This hands-on way of simulating variable and parameter ranges is uncommon in mathematical papers modelling immunotherapy. However, we believe such simulations are closer to the clinical situation, were heterogeneous patient population is treated, and this way of presenting mathematical modelling may be more tangible for physician scientists. This model is based on the assumption that T-cells lyse tumor cells repetitively throughout their life span. Therefore it is logical that a T-cell dose effect will be observed, but the extent of this effect could not be foreseen. However what is becoming evident by mathematical modelling and computer simulation is the degree to which cellular therapy needs to be augmented in order to expect a significant impact. The data clearly illustrates the quantitative effects for clinical trials: the T-cell dose ranged throughout all four studies from $1x10^8$ to $1x10^{11}$ T-cells. Assuming the lytic activity of the cells being equal, there was little chance of a

tumor response using Mackensen's approach, whereas the use of a 1000 times higher T-cell dose combined with synergistic measures (such as TBI prior to therapy) increased the likelihood of a response significantly. Delving deeper into Dudley et al.'s data the percentage of $CD8^+$ cells of TILs is consistently higher for responders than for non-responder patients (82.1% vs. 74.9% in the 2 Gy treatment, and 86.0% vs. 60.5% in the 12 Gy treatment). When comparing the percentage of specific Mart-1 of $CD8^+$ cells we find the same trend as before, as responder patients were administered with a higher percentage of Mart-1 cells (19.6% vs. 3.4% in the 2 Gy treatment, and 2.8% vs. 1.6% in the Gy treatment). Therefore, the responder patients clearly received higher dosage of effective Mart-1 cells. Therefore, taking a mechanistic view of the T-cell effect, the broad range alone can explain success or failure of the respective regimen. This model allows for the first time to compare unrelated studies with immensely varying treatment schedules.

Very little data is available on the exact numbers required for an efficient immune response against tumor antigens. As shown in Figure 3, simulation suggests the existence of a threshold of T-cell dose that has to be crossed before an effect on the tumor can be observed. The existence of such a threshold in a tumor T-cell therapy type of system was predicted by Kogan et al. (2009)[add ref no.]. Its clinical meaning is that the high renewal potential of the tumor renders any trial to just reduce the tumor and keep it in check, futile. Unless the tumor is eradicated no long-term stable equilibrium can exist. If the doses used are just below and above the threshold this effect will be most striking in the smallest tumors as can be seen in Figure 3.

In a recent work by Budhu et al. the efficiency of T-cell mediated killing has been carefully correlated with the T-cell concentration [30]. It is shown that the efficacy of such killing significantly increases, once the T-cell dose is beyond a certain threshold. In Budhu et al.'s work it is demonstrated that – in an in vitro setting - a ratio of 1000:1 to 10:1 of effector to tumor cells ensures the elimination of a tumor cell population of $1x10^4$ to $1x10^6$ respectively. When the researchers incubated melanoma B16 cells with T cells at roughly 1:1 ratio for 7 days and the presence of IL-2 they received nearly 100% killing. Without the presence of IL-2 the researchers required a 20:1 ratio to achieve tumor eradication and the functionality of the T-cells lasted for only 5 days. A distinct threshold value of T –cells is apparent below which the effect of T-cells is very weak and above which it is dramatic (Budhu et. al 2010, Fig.5). The

existence of a numerical threshold effect has recently been predicted in a mathematical analysis of the immunotherapy for treatment of glioblastoma [22]. Such stepwise function of immunotherapy is counter-intuitive for the physician – although not entirely unfamiliar when thinking of pharmacokinetics and pharmacodynamics. Identifying this step on the basis of mathematical modelling may increase the success rate of cellular immunotherapy.

In a clinical setting, a T-cell dose in the order administered by the Rosenberg group ($1x10^{11}$), which may be called a supraphysiological T-cell dose, may induce a cytokine storm – with release of INF-gamma, IL-8, TNF-alpha etc. Ultimately the inhibitory milieu of the tumor environment might be overcome, leading to upregulation of MHC-class I expression on the tumor cells and consequently increased tumor cell lysis.

In summary, mathematical modelling holds the promise that immunology – and especially human immunology is not just a black box, where the effects of a certain treatment are unpredictable, but that prior to a clinical trial the interplay between crucial parameters can be analyzed and predicted. The model is dependant on quantitative data: some of the parameters implemented in the model are deduced from diverse sources. The accuracy of model prediction can be improved, when such quantitative data are available for each individual study. It seems therefore necessary to raise the awareness of cellular therapists for the potential of mathematical modelling and improve the quality of quantitative data.

But even without exact data on tumor size and growth rate, we tried to account for biological variability by allowing a range of initial tumor sizes and growth rates. The efficacy of the T-cells – being regarded as “serial killers” in this model – may be lower than the values used for simulation, thus, if anything, required T-cell doses would even be higher.

Proliferation of tumor-specific T-cells may be crucial to solve the need for large amounts of T-cells as simulated in this paper. The proliferative capacity of antigen-primed T-cells depends on the differentiation state: late stage effector cells have a good lytic capacity but poor proliferative capacity and little in vivo persistence, whereas early effector memory T-cells or central memory T-cells have the capacity to further expand in vivo. Exciting new studies pinpoint towards differentiation pathways that can be manipulated ex vivo to maintain or induce a central memory phenotype,

thus increasing the persistence, functionality and the potential efficacy of such T-cells [31-33].

Biomathematical modelling of T-cell immunotherapy has its greatest value, when sufficient numbers of T-cells are available. In fact, we recently calculated for a glioblastoma model, that – technical challenges aside – a constant rate of T-cell infusions directly into the tumor bed over a prolonged time (weeks) may have the best effects [22]. Mathematical modelling can also predict the minimum number of T-cells needed to have a reasonable chance to have an impact onto the residual tumor masses. In other words, mathematical modelling can provide the order of magnitude of the T-cell treatment required.

We are aware of the technical challenges of generation and application of such large numbers of antigen-specific T-cells. However for some antigens such as Melan-A, using improved protocols, the T-cells can already be expanded to very high numbers within a short culture time [6], or – for antigens with a lower T-cell precursor frequency – large numbers of PBMC can be transduced to express modified and functionally improved tumor-specific T-cell receptors.[4] At the same time recent reports on severe adverse events following adoptive immunotherapy [7] as well as pre-clinical animal models showing significant toxicity after transferring TCR-gene transferred PBMC also emphasize the risks of T-cell immunotherapy and stress the need for meticulous pre-clinical evaluation including mathematical modelling. Mathematical modelling can help rationalize the design of either appropriate dose per patient, or alternatively determine exclusion criteria, for patients with large tumor burden in case large CTL (or TIL) dose is unavailable. In summary, mathematical modelling can help pick the right schedule for the right patient and thus hopefully improve treatment success.

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Figure legends:

Figure 1. A simplified model for the interaction of melanoma cells and cytotoxic T-cells and the respective cytokines. Melanoma cells express immunogenic antigens in the context of the major histocompatibility class I complex, but also secrete TGF-ß, which inhibits T-cell activity. Upon contact with melanoma cells, exogenously infused CTLs lyse their target and secrete IFN-γ. IFN-γ leads to upregulation of MHC-class I in the melanoma cells, which in return increases the CTL mediated effect. IL-2 injections prolong persitence of infused CTLs. Endogenous cytotoxic T-cells are inluded as constant influx to the T-cell effector compartment

Figure 2. Simulations of tumor cell number and TIL dose in the Khammari et al. (2007) clinical trial. (A-C) Simulations of time dependent tumor cell number dynamics of 100 random simulations are shown. Virtual tumors with randomly chosen initial tumor sizes (10^{10}-$5x10^{11}$ cells) and different growth rates (0,0001-0.001 h^{-1}) are simulated with (A) $0.85x10^{10}$ TILs given twice, one month apart. (B) $0.11x10^{10}$ TILs twice, one month apart, and (C) $1.35x10^{10}$ TILs twice, one month apart. Tumors that have reduced in size to practical zero (RECIST complete response, CR) are marked in red (thin solid line), tumors that have reduced by 30% (RECIST partial response, PR) are marked in blue (thick solid line). Tumors that have stabilized between 70% and 120% of their original size (RECIST stable disease, SD) are marked in green. Black (dotted line) marks time dependent tumor cell numbers simulations that were non-responsive, NR. The two ripples observed up to day 44 are due to the IL-2 treatment given for two weeks after each TIL transfusion.

Figure 3. Predictions of the final tumor cell number as a result of TIL dosage according to data derived from Khammari et al [23]. 100 virtual patients were simulated as in Fig 2 except that TIL dose was also randomly chosen from 5 different

dosages between $0.11x10^{10}$ to $1.35x10^{10}$, given twice, one month apart. Upper illustration shows simulated treatment results using the lowest TIL dose, middle illustration shows simulated results using intermediate TIL dose ($0.73\ x10^{10}$) and lower illustration shows simulated treatment results with the highest TIL dose. Red dashed line delineates the separation between responsive tumor sizes to its left, and non-responsive tumor sizes to its right.

Figure 4. Simulations of time dependent tumor cell number dynamics as a result of the Greenberg protocol (Yee et al. 2002) [25]. Infusions of $5.9x10^9$ ($3.3x10^9$ x 1.8 m^2 surface area of a human) were given at days 0, 14, 35, 56. Tumors that have reduced in size to practical zero (RECIST complete response, CR) are marked in red (thin solid line), tumors that have reduced by 30% (RECIST partial response, PR) are marked in blue (thick solid line). Tumors that have stabilized between 70% and 120% of their original size (RECIST stable disease, SD) are marked in green. Black (dotted line) marks time dependent tumor cell numbers simulations that were non-responsive, NR.

Figure 5. Simulations of time dependent tumor cell number dynamics as a result of the Mackensen et al. (2006) [24] clinical trial (A) and simulated improvement to the trial (B), (C) and (D). Simulations of time dependent tumor cell number dynamics of 100 random simulations are shown. Virtual tumors with randomly chosen initial tumor sizes (10^{10}-$5x10^{11}$ cells) and different growth rates (0,0001-0.001 h^{-1}) are simulated with (A) a presumed general Mackensen regimen ($2.1x10^8$ cell given 10 times at intervals indicated in the text). (B) All parameters the same as in (A) except CTL dose simulated here to be $5x10^8$ (A). (C) All parameter the same as in (A) except the CTL induced tumor cell kill rate is simulated here to be twice as in (A). (D) Combined effect of intensified regimens in (B) and (C). Tumors that have reduced in size to practical zero (RECIST complete response) are marked in red (thin solid line), tumors that have reduced by 30% (RECIST partial response) are marked in blue (thick solid line). Black (dotted line) marks time dependent tumor cell numbers simulations that were non-responsive.

Figure 6. Simulations of time dependent tumor cell number dynamics as a result of the Rosenberg intensive protocol [28]. (A) $1x10^{11}$ (multiplied by 0.1 assumed antigen

specificity ratio) antigen-specific T-cells given in a one time bolus infusion. (B) The same treatment as in (A) given to tumors 20% smaller than the initial tumor size in (A). (C) The same treatment as in (A) given to tumors 20% smaller than the initial tumor size in (A) and h_T larger by 33%. Tumors that have reduced in size to practical zero (RECIST complete response) are marked in red (thin solid line), tumors that have reduced by 30% (RECIST partial response) are marked in blue (thick solid line). Black (dotted line) marks time dependent tumor cell numbers simulations that were non-responsive.

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