PERIODICUM BIOLOGORUM
VOL. 114, No 2, 187–199, 2012

UDC 57:61
CODEN PDBIAD
ISSN 0031-5362

Essay

Towards Systems Based Immunotherapy of Cancer*

STANIMIR VUK-PAVLOVIĆ

College of Medicine, Mayo Clinic
Rochester, MN 55905, USA
E-mail: vuk@mayo.edu

Abstract

The essay, dedicated to Academician Drago Ikić–a pioneer in the development and preparation of vaccines for prevention of infectious diseases –at the occasion of his 95th birthday, discusses the relative failure of therapeutic vaccination for malignant diseases. From a personal perspective, the author recounts the historic examples of »spontaneous« tumor regressions in conjunction with acute infections; development of the first marketed purportedly antigen–specific cellular immunotherapy (for metastatic prostate cancer); the paradox of immunotherapy effect on overall survival, but not on time to disease progression; and the promise of the new complex systems–based approaches to a more rational design of cellular immunotherapy in the hope of bringing this mode of treatment into the therapeutic mainstream and standard of care.

Celebration of Academician Drago Ikić's remarkable life and achievements in prophylactic immunology and immunotherapy provides an opportunity to muse on the reasons why cellular immunotherapy of cancer is less remarkable. As a latecomer to the field I enjoy the benefit of hindsight and realization that the (empirical) quest to harness immunity in control of malignancy is centuries older than immunology itself (*cf.* ref. *35*). Yet, recognition that a tumor can be immunologically distinct from its tissue of origin is rather recent (*cf.* ref. *(11)*); it prompted the idea of immune surveillance, the mechanism whereby immunity constantly surveys the organism for damaged, diseased and malignant cells and eliminates them *(11)*. Hence, the rise of malignancy implies the downfall of immunity. As a result, immunity must be stimulated if it is to be counted on as a component of tumor therapy. Despite this rational underpinning, more than half a century of concerted effort to develop immune therapies has documented a history of failure that stands in sharp contrast with the magnificent achievement in prophylactic vaccination against infectious disease; those in immunotherapy of cancer stay humble in the shadow of the celebrant and his generation.

It is becoming increasingly clear that the reductionist views of the 20th century could not account for the complexity of interactions of the tumor and immunity, two evolving subsystems that exhibit selective pressure on each other. In distinction to antibiotics, (previously) considered as magic bullets against infectious agents, it is unlikely that immunological magic for cancer is on the horizon. (For an insightful

Received July 6, 2012.

* Essay published in the Festschrift for Academician Drago Ikić on the occasion of his 95th birthday.

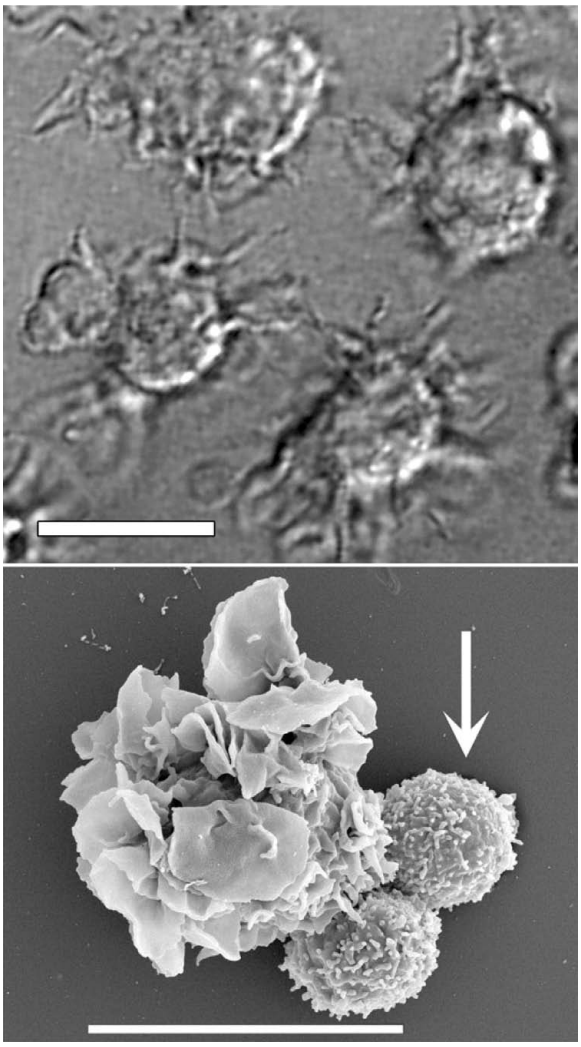

Figure 1. *Dendritic cells. Upper panel: Differential interference contrast micrograph of live human mature myeloid dendritic cells demonstrating characteristic protrusions; Lower panel: scanning electron micrograph of a dendritic cell interacting with two T cells (arrow). In both panels, bar=10 μm. From ref.* (23) © *the American Society of Hematology.*

view of the concept of the magic bullet, *cf.* ref. *(62)*). A major difference is that mechanisms of cancer immunotherapy are indirect, *i.e.*, unlike antibiotics they are not directed at cancer cells themselves, but at stimulating immunity to recognize and eliminate such cells. Thus, the required mechanism includes recognition of cancer and its elimination. The cells that can eliminate other cells cannot recognize the tumor on their own. First they must be »taught« to do so by specialized »teacher« cells. »Teaching« requires capture of the tumor cell (or, more generally, pathogens) and the subsequent clonal activation and expansion of tumor–specific effector cells (*cf.* any recent immunology textbook).

Boon of dendritic cells

Pathogen capture and processing are functions of innate immunity; stimulation of clonal expansion of immune effector cells is characteristic of acquired immunity. How the cells of innate immunity instruct the cells of acquired immunity was unclear until 1974 when Ralph Steinman and Zanvil Cohn reported the finding of the cell they named »dendritic« for the appearance of branched protrusions (Fig. 1) *(61)*. In the course of activation, dendritic cells (DCs) capture and process antigen and later, having lost that capacity, they acquire the ability to activate naïve and memory T (and other) cells (Fig. 1) in antigen–specific manner *(5)*. Later proven the most potent antigen-presenting cells hitherto discovered, dendritic cells have been actively studied, in large part because of their postulated use as immune therapeutics. This possibility gave rise to start-up companies developing technologies for dendritic cell preparation, »education« by antigen and testing in clinical studies.

It took almost twenty years to achieve the purported triumph in the quest to use dendritic cells as therapeutics. It came in 2010 as the approval in the United States of sipuleucel-T (Provenge®, Dendreon, Seattle, WA) for treatment of castration resistant metastatic prostate cancer. The event has been viewed as a milestone in development of cellular immunotherapy of cancer *(18)* that has reinvigorated interest in vaccination therapy of cancer, a field that earlier had lost its luster due in part to the absence of significant clinical progress and in part to the drying up of venture capital for funding early clinical trials. The new energy in the field has been recognized also by the 2011 Nobel Prize in Physiology or Medicine for the discovery of dendritic cells to Ralph Steinman, announced just days after his untimely death *(50)*.

The lesson of lymphoma

The long effort to clinical and commercial development of therapeutic cancer vaccines started with an earlier landmark, the success of dendritic cell vaccination of patients suffering from B-cell lymphoma. In 1996, Ronald Levy and colleagues at Stanford University treated four patients with patient's own dendritic cells »educated« by own monoclonal immunoglobulin receptor (monoclonal origin of the receptor results from the monoclonal nature of the disease) *(37)*. They selected the monoclonal protein as vaccination target based on the earlier demonstration in animal models that the idiotypic protein induced protective immunity against the tumor *(24)*. Importantly, the autologous protein was particularly immunogenic when presented by dendritic cells exposed *ex vivo* to that protein *(24)*. Levy and colleagues took advantage of that observation and prepared idiotypic proteins by culturing patients' lymphoma cells fused with a mouse–human heterohybridoma cell line *(13)*.

At that time, Levy's Stanford University colleague Edgar Engleman had already established methods for isolation of dendritic cells from peripheral blood and their *ex vivo* cultivation *(49)*. Cell isolation was largely based on density gradient centrifugation of leukapheresis product, the method Levy and colleagues employed in their study. From the blood of each patient they prepared a monocyte–depleted cell suspension and incubat-

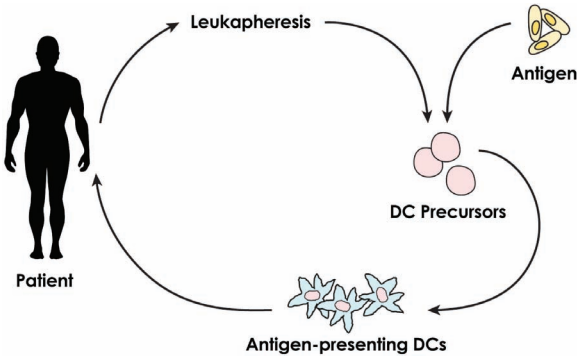

Figure 2. *General scheme of dendritic cell immunotherapy of cancer. White blood cells are isolated by leukapheresis and further fractionated to enrich the desired cell population. Most often dendritic cells are prepared from monocytes. Monocytes (or other cells) are incubated with a combination of cytokines and other molecules as differentiation agents and with tumor–associated antigens to generate activated antigen–presenting dendritic cells. These cells are infused or, more often, subcutaneously injected into the patient.*

ed it with autologous idiotype protein for two days. Treatment schedule included three monthly cycles of the procedure and one cycle five to six months later; the cycle consisted of leukapheresis, two-day exposure to antigen and infusion *(37)* (Fig. 2). Based on evidence from an animal model *(24)*, in each cycle immunity was boosted by subcutaneous injections of the soluble idiotypic protein. The results were impressive as all four patients developed cellular immunity to the idiotypic protein accompanied by positive clinical effects: one patient was in complete clinical remission for 21 months, one remained stable without further therapy, while the other two enjoyed more limited benefit *(37)*.

A company is born!

Concomitant with Levy's work, in 1992 Engleman and Samuel Strober, also of Stanford University, founded Activated Cell Therapy, the company aiming to develop dendritic cell based cancer therapeutics. The company chose to develop therapy for castration–resistant prostate cancer by raising immunity to prostatic acid phosphatase. Immunotherapy ought to have fulfilled the need for therapy specifically at the disease stage when circulating prostate specific antigen (PSA) levels increase despite androgen suppression while there is still no pain from metastases. Among the hoped for advantages of immunotherapy was the absence of major adverse events that could compromise quality of life otherwise acceptable during that stage of disease.

To develop marketable products, Activated Cell Therapy introduced significant technology changes compared to the technology used by Levy and colleagues. The aim was to simplify dendritic cell isolation and combination with antigen. One change was the introduction of cell–trap centrifugation on a density gradient. The method uses a patented disposable cell that fits into standard blood bank centrifuges and traps particular cells following centrifugation (Fig. 3). The additional feature of the method is the use of density gradients created by suspended colloidal organosilanized silica; compared to more traditional density gradient media, organosilanized silica provides higher stability and longer shelf life. Finally, the company developed the »antigen delivery cassette«, the fusion protein comprised of the antigen and a molecule with preferential binding to dendritic cells. In this project, the antigen has been prostatic acid phosphatase (PAP) and the DC-binding molecule the granulocyte-macrophage colony-stimulating factor (GM-CSF; refs. *(9, 58)*).

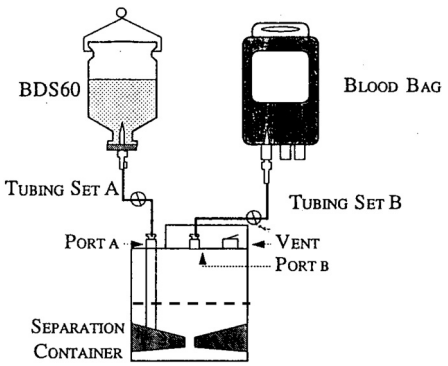

Figure 3. *Cell trap used to prepare sipuleucel-T. The separation container is divided by the septum (black) into the lower and upper chamber. The lower chamber and the lower part of the upper chamber above the septum (indicated by the dashed line) are filled with colloidal organosilanized silica (here designated as BDS60). The cell suspension contained in the blood bag is applied on top of the silica and the device is centrifuged. Low-density cells concentrate at the interface between the silica and cell medium in the upper chamber, while higher density cells are retained in the lower chamber. Modified from Fresenius DACS™ 300 instructions leaflet.*

Early clinical trials brought hopes of success

The hype encircling dendritic cells in the late 1990s obscured the fact that the cells prepared by the Activated Cell Therapy (later renamed »Dendreon«) protocol contained no more than one fifth of the cells characterized as dendritic (bright in CD54 *(58)*, but also in CD40, CD86, HLA-A,B,C and HLA-DR) while the rest were predominantly $CD3^+$ T cells combined with monocytes ($CD14^+$) and B cells ($CD19^+$; ref. *(9)*). Cell suspensions incubated with the PAP–GM-CSF construct were tested in two clinical trials. One, at University of California San Francisco, administered to patients the cells on three occasions four weeks apart; patients who displayed stable disease on week 24 received the fourth infusion *(59)*. The other protocol, at Mayo Clinic in Rochester, Minnesota, employed two infusions of cells one month apart, but followed by three injections of the soluble PAP–GM-CSF construct *(9)* based on earlier evidence from animal models *(24)* and the analogy with the study of B-cell lymphoma *(37)*.

The results from Mayo Clinic were published in June 2000 *(9)*. The goal of the phase 1 study was to determine

treatment safety and, as secondary endpoint, induce therapeutic immunity against prostate-related tissues in patients suffering from progressive castration-resistant metastatic prostate carcinoma. Altogether thirteen patients received two autologous antigen-presenting cell infusions and three injections of the soluble synthetic antigen. Patients were divided into three groups, each receiving a different dose of antigen per injection. Patients experienced mostly mild adverse events as short-lived fever and/or chills, myalgia, pain, fatigue or mild local reactions to soluble antigen injection. In three patients the levels of circulating PSA dropped as a result of therapy. In addition, therapy induced T cells that could be activated by GM-CSF and PAP *in vitro*; this demonstrated that immune tolerance for GM-CSF and PAP had been overcome. Injections of soluble antigen had no effect on T cells, but boosted the titer of antibodies to GM-CSF and, to a lesser extent, PAP. This study found no clinical response to treatment, but concluded that dendritic cells exposed to antigen *ex vivo* could induce antigen-specific cellular immunity in prostate cancer patients. These findings warranted further studies.

The San Francisco study came out in December of the same year *(59)*. It was a combined phase 1 dose-escalation study and a phase 2 study testing up to four administrations of the cellular product without injections of soluble antigen. The study included 31 patients altogether. The goals of phase 1 were identical to the study at Mayo; phase 2 looked for evidence of a delay of progression of disease. As at Mayo Clinic, patients tolerated treatment well with similar mild adverse events. A proportion of patients similar to that at Mayo developed immune response to the recombinant antigen and experienced a drop in circulating PSA levels. Significantly, treatment extended the time to disease progression in a manner positively correlated with the level of immune response to PAP and the dose of dendritic cells. These findings infused additional enthusiasm and energy into this exciting project and resulted in continued studies at the two institutions.

More good news

In the meantime, Levy's team continued the studies in B-cell lymphoma. In 2002 they published the follow-up to their 1996 paper; it included additional 31 patients *(65)*. Generally, the study confirmed earlier results *(37)* of durable objective tumor regression and induction of T-cells and antibodies reactive with autologous idiotypic protein. Interestingly, in patients resistant to DC vaccination or relapsing after it, the authors could induce complete tumor regression by injections of soluble idiotypic protein coupled to keyhole limpet hemocyanin. This finding seemed to have given additional credence to the protocol at Mayo Clinic employing injections of soluble PAP–GM-CSF to boost immunity against prostate derived tissues.

In 2004 Mayo Clinic reported the results of the phase 2 study that enrolled 21 patients who received the cellular product and soluble antigen *(10)*. Two patients exhibited a transient decrease in the levels of circulating PSA. However, another patient exhibited the reduction of PSA levels from the high levels of 221 ng/mL to the levels beyond detection. Even more impressive was the resolution of his metastatic retroperitoneal and pelvic adenopathy (Fig. 4) *(10)* and the fact that he was alive and free of disease nine years later (personal communication by Dr. Patrick Burch, Mayo Clinic). Despite an intense search for the specifics of this patient in comparison to those who did not respond, the only difference was that his peripheral blood mononuclear cells could be stimulated *in vitro* with soluble PAP–GM-CSF considerably longer than the cells of other patients. Comparison of the Mayo Clinic results with those from University of California San Francisco *(59)* demonstrated that immune and clinical effects of subcutaneous injections of PAP–GM-CSF did not differ from the effects of cell infusion alone *(10)*, contrary to the findings in mice *(24)* and patients suffering from B-cell lymphoma *(65)*. For that reason, further studies were conducted with the cellular product alone.

Mice are not little humans

The reasons for the observed discrepancy in the effects of soluble antigens on immune response and effect on malignancy between mice and humans remain unclear. For a long time I questioned the utility of comparing experimental murine tumor models and human cancer; mice are highly inbred, they are kept in pathogen-free conditions, live up to two years and usually are no older than two months when inoculated with the tumor. Commonly studied transplantable tumors have been rendered extremely aggressive by numerous passages that result in survival of the fastest growing cells. On the other hand, humans are genetically individualized, carry immunological memory of personal history of inflammation and infection, are differently physiologically »exhausted« by age and disease, and develop tumors that become manifest after many years of subclinical development *(26)*. Svetomir Marković, my colleague at Mayo Clinic, started looking into the relevance of murine models and found, for example, that murine and human dendritic cells responded differently to vascular endothelial growth factor (VEGF), a molecule often borne by tumors and implied in mechanisms whereby tumors evade immune surveillance; VEGF, highly immunosuppressive in humans is devoid of such effect in mice *(7)*. Consequently, a cancer immunotherapy protocol can be greatly more successful in murine models than in humans. It is thus feasible that such interspecies differences contribute to difficulties encountered in translation of immune therapy developed in mice into human clinical practice.

Towards the proof of efficacy

Individual clinical centers commonly lack the infrastructure and means to conduct large scale phase 3 clinical trials on their own. Therefore, usually such studies are conducted by institutional consortia. The first such

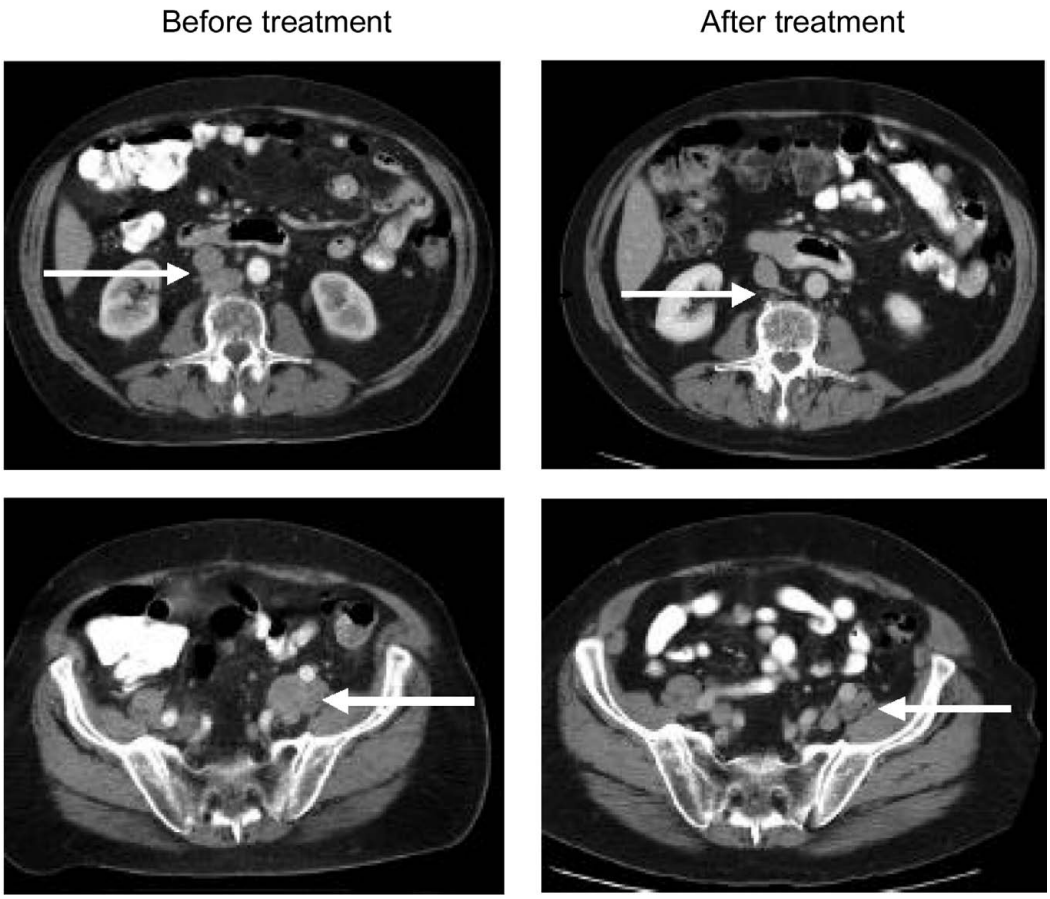

Figure 4. *Sipuleucel-T effect on metastases in the prostate cancer patient who underwent a complete clinical response. Left, computerized tomography scans obtained before treatment and, right, 22 months after initiation of treatment. Arrows indicate the sites of retroperitoneal lymph nodes (upper panels) and pelvic lymph nodes (lower panels). From ref.* (10) *© John Wiley & Sons, Inc.*

study of the cellular product (now bearing the generic name sipuleucel-T and commercial name Provenge®), rather limited in scope, came out in July 2006 *(59)*. The primary clinical endpoint of the study of 127 patients was the time to disease progression (determined by radiological observations, onset of pain, neurologic changes, *etc*.). It was rather sobering that treatment conferred no delay in the time to progression, but the overall survival was extended by 4.5 months. Based on this evidence, Dendreon Corporation applied to the U.S. Food and Drug Agency for the license to market the product. To the disappointment of many and furor of others, the Agency refused licensure on the grounds that retrospective data on survival are not sufficient evidence of activity and requested further prospective studies *(3)*. Consequently, a parallel ongoing phase 3 study was reorganized with survival as the primary endpoint *(32)*. The study included a control group of patients who also underwent three therapy cycles two weeks apart, but in each cycle they received only one third of cells processed without PAP–GM-CSF (compared to treatment group that received the full amount of cells prepared with PAP–GM-CSF); the remaining cells were frozen for the possible later use.

At the time Mayo Clinic started prostate cancer studies in the late 1990s, our colleagues in the Division of Hematology decided to take advantage of the local ability to prepare autologous antigen presenting cells; they applied them to patients suffering from multiple myeloma *(46)*. Patients were treated while in remission after transplantation of autologous bone marrow. They received autologous antigen presenting cells exposed *ex vivo* to autologous serum as the source of disease–characteristic monoclonal protein. Time to progression was the primary endpoint. Disappointingly, treatment had no effect on progression. Some years later, prompted by evidence of extended survival in prostate cancer studies *(60)*, patient data were revisited; it was found that patients treated by immunotherapy exhibited the median survival of 5.3 years compared to 3.4 years for patients who had not received immunotherapy *(46)*. Although the study did not include a control group, but the results were compared to historic controls, the data indicated the need for further controlled studies.

By the spring of 2010, Dendreon completed the definitive phase 3 study that confirmed the survival benefit observed in earlier studies and obtained the license on April 29 *(18)*. The data were published the following

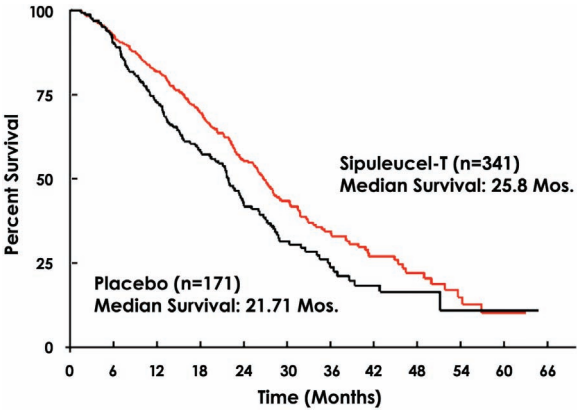

Figure 5. *Sipuleucel-T (Provenge) extends survival of prostate cancer patients as determined by a phase 3 study* (41). *The median survival benefit was 4.1 months. Modified from Dendreon promotional material (www.dendreon.com).*

July; the final study found „a relative reduction of 22 percent in the risk of death as compared to the placebo group … a 4.1 month improvement in median survival (25.8 months in the sipuleucel-T group vs. 21.7 months in the placebo group)" *(41)* (Fig. 5). It took 18 years from Engleman's and Strober's founding of Activated Cell Therapy to Dendreon's licensure of sipuleucel-T!

Is it just dendritic cells?

Yet, the questions about the actual mechanism of action of sipuleucel-T persist, mainly because of the paradoxical effect on overall survival without the effect on the time to progression. In addition, the very benefit of sipuleucel-T has been questioned on the basis of possible errors in the phase 3 study design. Examining the unpublished documents submitted to the U.S. Food and Drug Administration in the process of obtaining the marketing permit, it appeared that control patients above 65 years of age lived 11 months less than control patients younger than 65 *(38)*. This is surprising as such age difference is not normally observed in prostate cancer patients at the same stage of disease or in control groups of other (immuno)therapy studies. In addition, sipuleucel-T extended overall survival in older patients from 17.3 months to 23.4 months, while the difference in younger patients was less than one month. The authors speculate that older control patients tolerated the repeated leukocyte depletion less well than younger patients. Consequently, the overall survival of the entire control group was artificially reduced giving the impression of sipuleucel-T efficacy. This provocative finding needs to be tested.

Dendreon responded swiftly by a *post hoc* »exploratory« statistical analysis of the effects of PAP–instructed antigen-presenting cells (derived from the frozen two thirds of leukapheresis product) on the overall survival benefit *(29)*. Using the rank-preserving structural failure time model *(30)* they estimated »the treatment effect of sipuleucel-T relative to control had no crossover to [cells derived from frozen product] occurred.« Had the control subjects not been compassionately treated and assuming that the product of frozen cells was equally effective as the fresh product, the study concluded that sipuleucel-T extended overall survival by 7.8 months, i.e, almost twice compared to the results of original analysis of the same clinical cohorts *(29)*. Nonetheless, the study neither addressed the fact that control vaccination by one third of unprocessed mononuclear cells was mechanistically inadequate nor that the older control subjects lived less that would be expected if left untreated *(38)*.

The question of the mechanism has not been answered for other experimental immunotherapies either. What is the relationship of tumor–antigen–specific T cells in patients and clinical response? For a long time it has been a recognized yet unappreciated fact that tumor–specific T cells arise spontaneously *(31, 48)*. Thus, even in the absence of any immunotherapy immunity registers tumor cells, but fails to eliminate them (*cf.* ref. *(69)*). On the other hand, the only other major immune treatment of immunity is not antigen-specific at all: it is the intravesically applied *Bacille Calmette–Guérin* (BCG) for treatment of muscle non-penetrating bladder carcinoma *(61)*. Superficially, the two treatments are mechanistically different, yet there is no conclusive evidence that it is indeed so. The major problem is that the critical failing element (or elements) of immunity that allowed tumor development is unknown, as it is the required status of immunity if effective and predictable eradication of the tumor is to be achieved.

The clues into the resolution of the latter problem can come from sources such as the studies correlating immune parameters with survival. For example, the extent of infiltration of $CD8^+$ T cells into the tumor and the abundance of these cells and T regulatory cells in some cases can predict survival *(27, 52)*. This insight prompted the proposal of »immune score«, a quantifier of the presence of immune cells in the tumor *(52)* that can be used as a predictor of survival and tool for design of »combinatorial immune-based therapies that reduce tumor-associated immune suppression to unleash pre-existing or therapeutically induced tumor immunity« *(20)*.

Another lead came from Avrum Bluming and John Ziegler of the Uganda Cancer Institute. In 1971 they reported the case of an eight-year old boy diagnosed with Burkitt's lymphoma *(8)*. Ten days after diagnosis and before any treatment for lymphoma had been commenced, the boy developed skin rash characteristic of measles infection; the infection was subsequently confirmed by the appearance of measles-specific antibodies *(8)*. The authors reported that »over the course of the next two weeks both the exanthem and the tumour disappeared«. Although Bluming and Ziegler gave no definitive mechanistic explanation of the curative effect of measles infection, their dramatic observation has not remained unnoticed. Subsequent appreciation of the oncolytic properties of the measles virus, the safety of its vaccine strains and the role of immunity gave rise to the effort to use genetically modified measles virus as a means of experimental cancer therapy *(6, 39)*.

Nihil novi sub sole

The relationship among infection, immunity and malignancy has been recognized well before the advent of modern science. Between 1775 and 1980 there have been 449 documented cases of »spontaneous« tumor regressions in patients suffering from some concurrent acute infection *(16)*. These observations led to the development and use of nonspecific immune boosts such as Coley's toxins (a bacterial extract) in the early 20th century *(67)* and the efforts to engage immunity by adjuvants such as BCG *(66)* or purified bacterial products *(57)*. However, this mode of therapy mostly fell into oblivion reminiscent of the abandonment of bacteriophage therapy of bacterial infection *(56)* in the wake of development of antibiotics. It is plausible that the well known Lewis Thomas's notion that »the phenomenon of homograft rejection will turn out to represent a primary mechanism for natural defense against neoplasia« *(64)* was not only the impetus for the formulation of the theory of immune surveillance *(11)*, but also for the push of nonspecific immunotherapy into (at least temporary) oblivion. Yet, it turns out that »nonspecific« mechanisms can act, in part at least, by affecting antigen presentation by dendritic cells *(40)*.

A notion inherent in the concept of immune surveillance is that immunity »searches« for particular signatures of cancer that can be characterized in molecular terms. In concert with the dominating reductionist and particularistic approach of biomedical science of the 20th century, the ensuing scientific advances have focused in large part on the molecular characterization of cancer cells and definition of molecular targets of immune attack (*cf.* refs. *(68, 69)*). (For an enlightened and entertaining critique of reductionism, see ref. *(42)*). This effort has been quite successful as it resulted in characterization of numerous tumor–associated epitopes (*cf.* ref. *(19)*). Yet, manifestation of malignancy implies—in contrast to the acute phase of an infection by a single microbe population—that an entire regulatory system failed. Far from trivializing the effort and success of therapies based on particular molecular targets, it does appear that the effort towards more effective cancer treatments must be based on insights facilitated by systems approaches *(28)*.

The riddle of survival benefit

The finding that sipuleucel-T affects survival, if it does *(38)*, but not disease progression is not unique. Other examples include similar observations in post-transplant multiple myeloma *(46)* and a phase 2 study investigating the effects of an allogeneic prostate cancer whole-cell vaccine *(51)*. In the latter study, castration–resistant prostate cancer patients were vaccinated by 14 subcutaneous administrations over a year. The vaccine consisted of three lethally irradiated allogeneic human prostate-derived cell lines; the first two administrations contained also BCG. The study was not controlled, *i.e.*, the results were compared to historic data, rather than to a group concomitantly treated by placebo. Interestingly, the study found that vaccination doubled the time to disease progression »as defined by clinical and/or radiologic criteria« *(51)*. The patients responded also by slowing the rate of PSA level increase (»PSA velocity«) and exhibited a more pronounced T_h1 (cell–mediated cytotoxic) character of immunity compared to non-responders. The ensuing controlled phase 2 study, using time to progression as endpoint again, failed at interim analysis (*i.e.*, continuation of the study had no chance of achieving statistical significance) and was discontinued. However, years later an *a posteriori* analysis demonstrated that patients in this trial lived six months longer compared to placebo treated controls (personal communication from Dr. Anthony Walker, Alacrita Consulting, London, England).

Chemotherapy may not be the right paradigm for immunotherapy

The paradox of long-term, but not short-term benefit of vaccination therapy is supported by observations from other cancer vaccination studies (*cf.* ref. *(22)*). Many studies were discontinued when interim analysis indicated that the final endpoint could not be reached, yet subsequent–often anecdotal–evidence indicated survival longer than expected. Common to all these studies has been the use of statistical methods developed for studies of chemotherapy where treatment effects are generally observed rather promptly. A characteristic of these methods is that they do not take into account the delayed separation of survival (Kaplan–Meier) curves for treatment and control groups observed in immunotherapy. Consequently, there have been efforts to define a »methodological framework to enhance the clinical success of cancer immunotherapy« *(34)* and overcome the »critical hurdles in cancer immunotherapy« *(25)*.

A major issue in clinical studies of dendritic cell vaccines has been the lack of standards for their preparation and administration. Dendritic cell differentiation, antigen capture and processing and consequent presentation are complex; depending on the differentiation stage, a dendritic cell can induce tolerance to an antigen or stimulate immunity against it *(5)*. Cell dose, route of administration, number of vaccinations, and the need for adjuvants are just the few among parameters that vary from one clinical trial to another *(21)*. It is not surprising that the need for standardization has been considered quintessential for further progress. In 2004, Carl Figdor and colleagues considered and recommended for standardization the parameters pertinent to vaccine preparation according to current Good Manufacturing Practices, vaccine quality control, patient characteristics, trial design, documentation and definition of clinical response, description of clinical outcome, description of immune parameters before and after vaccination and assays proposed to measure these parameters *(21)*. A particular problem has been standardization of the required immune parameters as it is still not clear which parameters reflect the tumor–specific immune function and what that function needs to be for effective tumor therapy (*cf.* ref. *(20)*). It is evident that such standardization is difficult among numerous early clinical studies conducted

worldwide. On the other hand, the (mostly industry sponsored) multi-institutional phase 3 trials can be standardized more easily as they are centrally designed and controlled. Alas, in the still rare phase 3 studies of cellular immunotherapy the study sponsors are generally interested in funding just the clinical aspects of the study. For example, a MedLine search on March 20, 2012 using keywords »Sipuleucel-T« or »Provenge« had 161 hits; aside from the aforementioned clinical studies with limited monitoring of immune function, not a single one deals with immune mechanisms underlying the observed clinical effects! There is little surprise that a *Nature Biotechnology* editorial scolds Provenge as »a success for clinical and manufacturing brawn over molecular precision« *(4)*.

More recently, slow progress to clinical success has been attributed to the absence of realization that the »methodological framework for immunotherapy development … is distinct from that widely used for chemotherapy« *(34)*. The proposed framework for immunotherapy includes a new paradigm of distinct steps – from the proof of principle through toxicity screening, measurement of biologic activity of the vaccine, measurement of immune response in clinical trials, dose and schedule optimization, setting developmental decision points, trial design and clinical endpoints. Further, specific clinical kinetics of immunotherapy must be taken into account by adjusting endpoints accordingly and by developing appropriate statistical tools *(34)*.

The broad interest in overcoming the current obstacles to progress of immunotherapy has resulted in consensus – forging meetings, such as a recent one by the Society for Immunotherapy of Cancer. Its recommendations focused on problems related to extrapolation from murine models to humans, delays due to administrative hurdles (institutional, administrative and regulatory), cancer biology (tumor complexity and heterogeneity, mechanisms of tumor escape from immunity), limited access to reagents and funding as well as insufficient number of multidisciplinary teams of scientists and clinicians. Of note, the report focuses on two fundamental problems: lack of markers of immune response and inadequacy of conventional criteria of clinical response for immunotherapy *(25)*.

Complex systems and simple views

Given the enormous complexity of the system and numerous unresolved issues, is there a way to a more rational design of effective cancer immunotherapy? A new promise arises from considerations of immunotherapy beyond the dose–response paradigm (essentially the scientifically unjustified translation of the Newtonian action–reaction principle into immunotherapy), but in terms of complex systems biology. One example is the consideration of the role of drug administration schedule and timing on cytotoxic *(35)* and immune *(53)* effects. Twenty years ago, Zvia Agur noticed that normal cells, but not malignant cells, divide at a rather constant rate and developed a mathematical model of the effect of cytotoxic drugs in a system composed of normal cells and malignant cells *(15)*. She found that delivering a (short-lived) cytotoxic drug at the frequency coinciding with the period of normal cell division reduced toxicity to these cells (*e.g.*, hematopoietic precursors) while retaining toxicity for malignant cells. This finding was confirmed contemporaneously in an animal model *(1)*. Such studies indicate the benefit of expanding therapeutic considerations beyond just the intended target cell population.

A more recent example took advantage of the observation that the levels of circulating C-reactive protein (CRP) could be used as a measure of immune activation *(17)*. Based on the much more frequent than usual measurements in patients suffering from advanced malignancies, it was discovered that CRP levels fluctuate cyclically with the average period of some seven days. This observation led to the hypothesis that the oscillations reflect the time-dependent interactions of immune activation (*e.g.*, rise of T_h1-type effectors) and immune attenuation (*e.g.*, increase in the levels of T_{reg} cells; ref. *(17)*). Consequently, the authors postulated that the effects of immune treatment depended on synchronization with CRP oscillations, *e.g.*, the effect of a cytotoxic drug will be different if administered at the peak of T_h1 activity or of T_{reg} cell levels, in accord with Dr. Agur's suggestion many years earlier for cytotoxic therapy *(1, 15)*.

This hypothesis has been recently tested in eleven patients suffering from unresectable malignant melanoma and treated by temozolomide, a lymphocyte depleting cytotoxic drug *(47, 54)*. Together with the C-reactive protein and progression-free survival, 29 humoral and 22 cellular markers of immune functions were measured as a function of time and analyzed by a specially developed algorithm. The results demonstrated oscillations in the ratio of polarized macrophages (M1/M2), cytokine IL-17, $CD11c^+/CD14^+$ cells, *etc*., mostly with periods of 3, 6, 9 and 12 days. While the small number of subjects makes the results still preliminary, it is interesting that progression-free survival was related to the first derivative of the fitted function on the day of treatment. For example, progression-free survival was longer when the cytotoxic drug was administered during a cyclical increase in the levels of IL-12p70 or decrease in the ratio of M1/M2 macrophages. More extensive clinical testing of this exciting hypothesis is underway. (For an insightful view on this approach, see ref. *(33)*).

Is it all in the timing?

Oscillations in tumor–immunity interactions have been studied within the context of systems theory. For example, a rather simple model posits that the number of tumor cells oscillates in time with amplitudes and dampening characteristics dependent on tumor–associated antigens and level of immunity *(43)*. Assuming that the mathematical model is a reasonably accurate quantitative description of the biological system, one can computationally vary the parameters that measure antigen »strength« and level of immunity and simulate the changes in tumor

burden under particular conditions and predict conditions that will lead to tumor eradication *(43)*. For therapy by a single immunomodulator (*e.g.*, cytokine IL-2), curative doses can be unacceptably toxic. Conceivably, this problem can be mitigated by combination of several therapeutic agents, each at the acceptable dose level.

Rational design of a combination therapy protocol that minimizes toxicity and maximizes efficacy can be complex. For example, by the application of optimal control theory one can search for the best strategy of combining treatments by adjusting their dose and/or administration schedule. The results of a study simulating discrete administrations of cytotoxic T cells and IL-2 in treatment of an *in silico* tumor demonstrate the benefit of the optimized dose and schedule of each agent separately and of treatment by both according to a protocol that takes into consideration efficacy, toxicity, and clinical feasibility of administration *(12)*. This paradigmatic study and many similar ones demonstrate the potential of computational assessment of the effects of a particular therapeutic approach under ideal(ized) conditions.

Proof is in the patients

Rarely, if ever, have clinical protocols been designed with the input of mathematical biologists and even less frequently have their concepts been tested in prospective clinical trials. For that reason it is impossible to tell whether mathematicians' attempts to model tumor immunotherapy are much more than intellectual diversion or desperate attempts at relevance. Hence, some have taken the opposite approach, testing new mathematical formulations against existing clinical data. Surprisingly, despite the glut of clinical trials testing a myriad of treatments, there are preciously few reports of clinical data measured with sufficient precision and frequency to make testing of mathematical models possible. A particular problem is the quantitative assessment of tumor burden. One way to partially overcome this problem is to use surrogate markers, *e.g.*, PSA in metastatic prostate cancer (however imperfect and misleading it probably is; *(55)*).

The common approach in cancer therapy modeling has focused on the description, quantification and consequent prediction of effects in patient populations. Because patients respond to prostate cancer vaccination differently *(51)*, it is questionable whether mathematical modeling can predict the effects of immunotherapy for individual patients. To address this question, Kronik and colleagues formulated a mathematical model of basic interactions among the therapeutic vaccine, immune system and prostate cancer cells *(45)*. The simplistic mathematical model (Fig. 6) was established on the implication that the cellular vaccine interacts with DC precursors maturing them into antigen-presenting DCs that migrate from skin into the lymph node. Antigen–presenting DCs induce T_h1–type immunity resulting in tumor–specific cytotoxic cells that kill tumor cells. Eventually, antigen–presenting DCs give rise to regulatory DCs that recruit regulatory/inhibitory cells that counter the activity of tumor–killing cells. Prostate cancer cells propagate

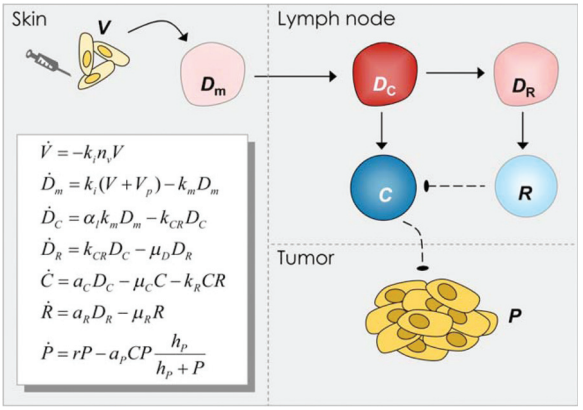

Figure 6. *Model of simplified interactions of immunity and prostate cancer (P). V, cellular vaccine; D_m, antigen presenting dermal dendritic cells; D_C, mature dendritic cells; D_R, "exhausted" dendritic cells; R, regulatory/inhibitory cells; C, antigen–specific effector cells (e.g., cytotoxic T cells). From ref.* (44).

exponentially, while the rate of tumor–cell killing is proportional to the number of cytotoxic cells. Vaccination was modeled as instantaneous addition of the vaccine at each injection time.

Using kinetic parameters extracted from the literature, the model was tested on rather extensive PSA velocity measurements taken over a year (at each of 14 vaccine administrations) for each individual patient in the aforementioned clinical study of an allogeneic prostate cancer whole-cell vaccine *(51)*. (PSA was used as a quantitative correlate of cancer burden in the absence of a more pertinent marker and as an indicator of acute perturbation of the tumor by therapy.) The models successfully described PSA levels measured for individual patients before and during the initial five to nine treatment cycles and yielded the values of patient-specific parameters *(45)*; this process resulted in an individualized model for each patient. Individualized models successfully predicted the PSA course during subsequent vaccination cycles in 12 out of 15 patients responding to therapy (Fig. 7). Thus, the model has been validated, *i.e.*, found that it described the system within the constraints of its assumptions and limits of the data *(45)*. It also means, that – within the same constraint and limits – it had the power of prediction.

As in all patients the disease eventually progressed *(51)*, the model was used to predict whether a change in vaccine dose or administration schedule would lead to more favorable clinical outcome. The goal was to stabilize PSA levels from the moment of model validation to the planned end of treatment (12 months since inception). Simulations of the PSA course indicated that patients differed in the requirement for dose or schedule modification (Fig. 8) *(45)*; for some, the required modification was modest (*e.g.*, doubling the frequency of administration), while for some even the maximal clinically feasible intensification of dose or schedule would not suffice. Consequently, such patients could be directed to different treatment modalities.

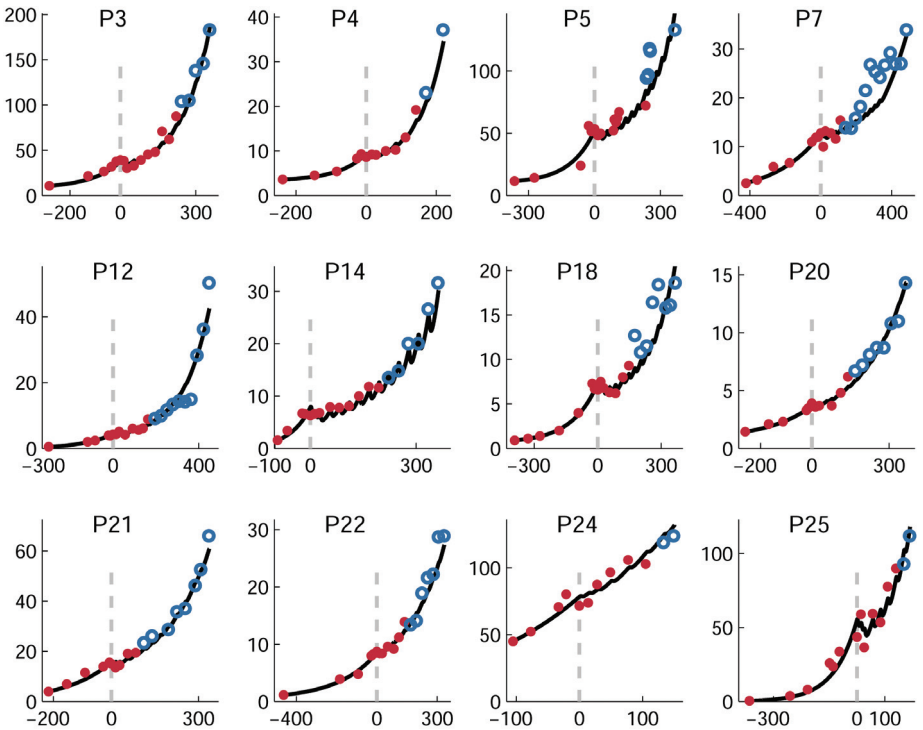

Figure 7. *Validation of individualized models for patients responding to vaccination. Patient-specific best-fit model parameters were derived by fitting the model to the respective pretreatment PSA values and the initial in-treatment PSA values (red). Subsequent PSA levels (blue) were predicted by the use of the obtained best-fit parameters. In this figure and Fig. 8 vertical dashed lines indicate the beginning of vaccination treatment on day 0. Achieving good predictive power required a different size of the training set for each patient. From ref.* (45).

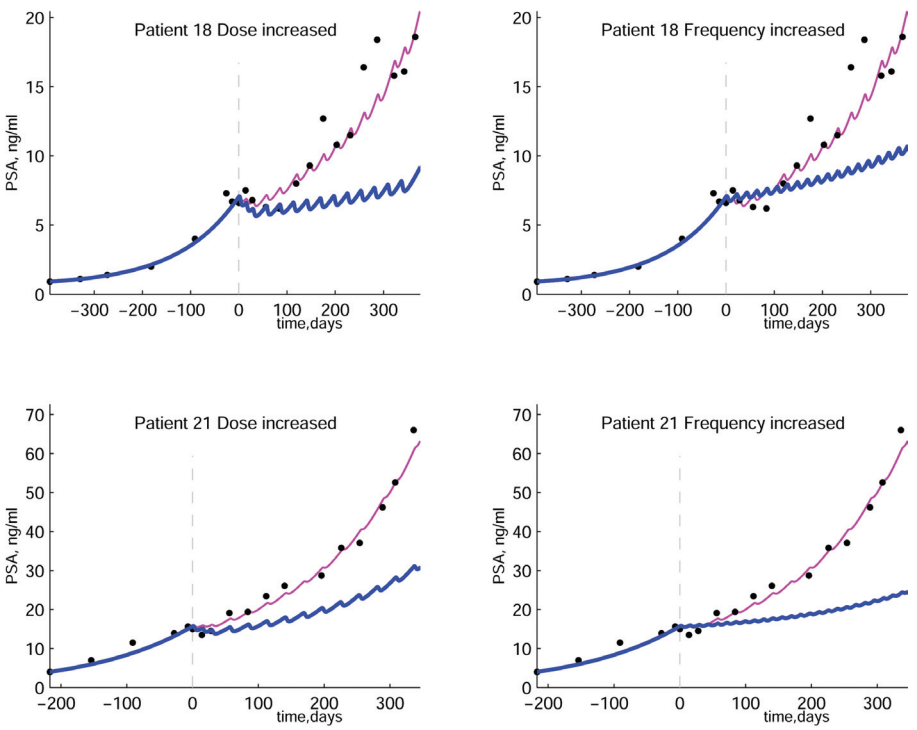

Figure 8. *Stabilizing PSA levels by model-aided modification of the vaccination regimen. Individualized models for Patients 18 and 21 were used to predict PSA dynamics after modification of the vaccination regimen within limits deemed clinically possible. Red lines represent the best-fit curves to PSA dynamics observed under the standard treatment regimen (2.4×10^7 vaccine cells administered every 28 days); blue lines are the predicted courses of PSA levels when vaccination regimens is modified. For Patient 18, the simulated effects are shown of the doubling of vaccine dose or reducing the vaccination interval to 21 days. For Patient 21, the vaccine dose was tripled or vaccination interval halved. From ref.* (45).

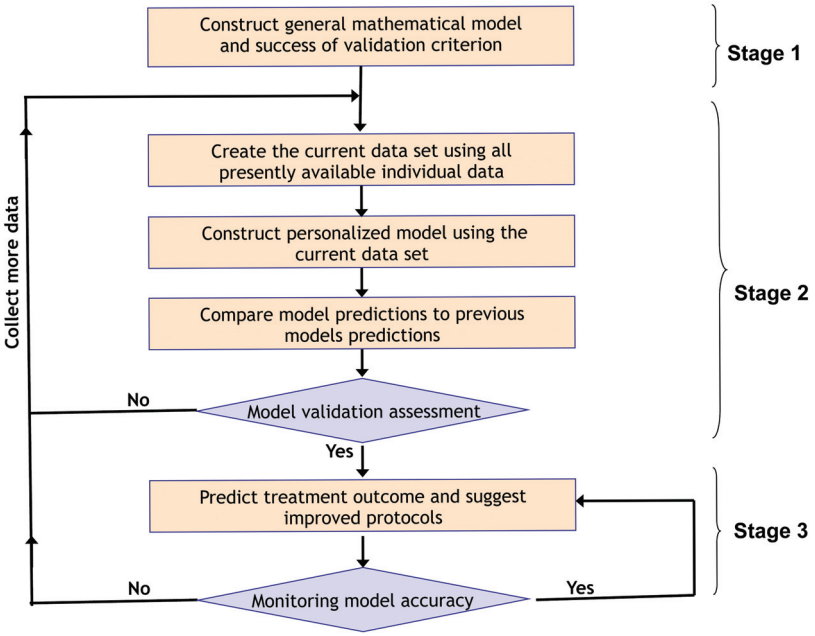

Figure 9. *At Stage 1, a general mathematical model and the success validation criteria are designed, based on available preliminary data and biological insight. At Stage 2, new personal clinical data are collected and model personalization and validation assessment are repeated until the success of validation criterion is satisfied. Then, at Stage 3, the validated model is used to predict treatment outcomes and suggest treatment modification.*

Into the future

Though still only hypothetical and clinically untested, *in silico* success of mathematical models in predicting the effect of treatment and guiding its personalized optimization led to the conclusion that clinical studies in immunotherapy (and possibly other treatment modalities) of cancer require a novel approach if immunotherapy is to enter the mainstream of cancer treatment any time soon *(2)*. This will require a new and higher level of collaboration of mathematicians, basic and translational scientists, and clinicians who will jointly plan and design novel clinical studies. These clinical trials will have to allow for personalized treatment (»P-trials«) that is currently beyond the format recognized by regulatory authorities. In the future, authorities will have to permit personalized doses and schedules within a restricted range (instead of the currently standard testing of the population response to a fixed dose and schedule).

To facilitate this transition, we proposed a novel computational algorithm that allows mechanism–based real–time dynamic treatment personalization (Fig. 9) *(44)*. Briefly, based on the available preliminary data and biological evidence, one designs the general mathematical model and criteria for success of its validation. Subsequently one accumulates personal clinical data as treatment progresses and repeats model personalization and assesses its validation until the criterion of validation success is satisfied. Then one employs the validated model to predict treatment outcomes and suggest treatment modification *(44)*. If successful in the clinic, the method will allow response–based adjustments of vaccine dose and/or administration schedule in the course of treatment and will be useful in stratifying patients with respect to the likelihood of clinical response. As the methods for noninvasive measurements of tumor burden become more available and precise, this approach will be more easily testable.

Epilogue

The vast effort invested into immunotherapy of cancer over the past century has not paid off yet; it has not made immune treatment definitive and has not brought it to the standard of care. Here I mused on the increasing understanding of malignancy not as analogous to infection, but as system failure. This understanding is starting to yield results. Systems–based approaches are leading to the appreciation of the role of the dynamic nature and evolutionary character of interactions of malignancy and the patient. In that, we are becoming increasingly aware of the role of individual history of infection and immunity, age, individuality of the tumor, and the dynamism of their interactions. We are progressively less puzzled by the centuries-old and some recent reports of »miraculous spontaneous« cure of cancer and are starting to understand mechanisms linking acute infection, immunity and cancer. The hope seems to be well founded that the current generation of physicians and scientists will accomplish in cancer immunotherapy a level of success commensurate to that by Academician Ikić and his generation in immunization directed at control of infectious disease.

Acknowledgements: *I thank Professor Zvia Agur and Dr. Yuri Kogan for critical reading of the manuscript.*

REFERENCES

1. AGUR Z, ARNON R, SCHECHTER B 1992 Effect of the dosing interval on myelotoxicity and survival in mice treated by cytarabine. *Eur J Cancer 28A:* 1085-1090
2. AGUR Z, VUK-PAVLOVIĆ S 2012 Mathematical modeling in immunotherapy of cancer: Personalizing clinical trials. *Mol Therapy 20:* 1-2
3. ANONYMOUS 2008 The regulator disapproves. *Nature Biotechnol 26:* 1
4. ANONYMOUS 2010 An empiric victory. *Nature Biotechnol 28:* 529
5. BANCHEREAU J, STEINMAN R M 1998 Dendritic cells and the control of immunity. *Nature 392:* 245-252
6. BLECHACZ B, RUSSELL SJ 2008 Measles virus as an oncolytic vector platform. *Current Gene Therapy 8:* 162-175
7. BLOCK M S, NEVALA W K, LEONTOVICH A A, MARKOVIC S N 2011 Differential response of human and mouse dendritic cells to VEGF determines interspecies discrepancies in tumor-mediated Th1/Th2 polarity shift. *Clin Cancer Res 17:* 1776-1783
8. BLUMING A Z, ZIEGLER J L 1971 Regression of Burkitt's lymphoma in association with measles infection. *Lancet 2:* 105-106
9. BURCH P A, BREEN J K, BUCKNER J C, GASTINEAU DA, KAUR JA, LAUS RL, PADLEY D J, PESHWA MV, PITOT HC, RICHARDSON R L, SMITS B J, SOPAPAN P, STRANG G, VALONE F H, VUK-PAVLOVIĆ S 2000 Priming tissue–specific cellular immunity in a phase I trial of autologous dendritic cells for prostate cancer. *Clin Cancer Res 6:* 2175-2182
10. BURCH P A, CROGHAN G A, GASTINEAU D A, JONES L A, KAUR J S, KYLSTRA J W, RICHARDSON R L, VALONE F H, VUK-PAVLOVIĆ S 2004 Immunotherapy (APC8015, Provenge) targeting prostatic acid phosphatase can induce durable remission of metastatic androgen-independent prostate cancer: A phase 2 trial. *Prostate 60:* 197-204
11. BURNET F M 1970 The concept of immunological surveillance. *Prog Exp Tumor Res 13:* 1-27
12. CAPPUCCIO A, CASTIGLIONE F, PICCOLI B 2007 Determination of the optimal therapeutic protocols in cancer therapy. *Math Biosci 209:* 1-13
13. CARROLL W L, THIELEMANS K, DILLEY J, LEVY R 1986 Mouse × human heterohybridomas as fusion partners with human B cell tumors. *J Immunol Methods 89:* 61-72.
14. CHEN T T, LEVY R 1995 Induction of auto-antibody responses to GM-CSF by hyperimmunization with an Id-GM-CSF fusion protein. *J Immunol 154:* 3105-3117
15. COJOCARU L, AGUR Z 1992 A theoretical analysis of interval drug dosing for cell-cycle-phase-specific drugs. *Math Biosci 109:* 85-97
16. COLEY NAUTS H 1980 The beneficial effects of bacterial infections on host resistance to cancer; end results in 449 cases. Cancer Research Institute, Monograph No. 8, New York.
17. COVENTRY B J, ASHDOWN M L, QUINN M A, MARKOVIC S N, YATOMI-CLARKE S L, ROBINSON A P 2009 CRP identifies homeostatic immune oscillation in cancer patients: A potential treatment targeting tool? *J Transl Med 7:* 102
18. DEFRANCESCO L 2010 Landmark approval for Dendreon's cancer vaccine. *Nature Biotechnol 28:* 531-532
19. DE SMET C, LURQUIN C, DE PLAEN E, BRASSEUR F, ZAROUR H, DE BACKER O, COULIE P G, BOON T 1997 Genes coding for melanoma antigens recognised by cytolytic T lymphocytes. *Eye 11:* 243-248
20. EMENS L A, SILVERSTEIN S C, KHLEIF S, MARINCOLA F M, GALON J 2012 Toward integrative cancer immunotherapy: Targeting the tumor microenvironment. *J Transl Med 10:* 70 (doi: 10.1186/1479-5876-1110-1170).
21. FIGDOR C G, DE VRIES I J, LESTERHUIS W J, MELIEF C J M 2004 Dendritic cell immunotherapy: Mapping the way. *Nature Med 10:* 475-480
22. FINKE L H, WENTWORTH K, BLUMENSTEIN B, RUDOLPH N S, LEVITSKY H, HOOS A 2007 Lessons from randomized phase III studies with active cancer immunotherapies: Outcomes from the 2006 meeting of the cancer vaccine consortium (CVC). Vaccine 25: B97-B109
23. FISHER P J, BULUR P A, VUK-PAVLOVIĆ S, PRENDERGAST F G, DIETZ A B 2008 Dendritic cell microvilli: A novel membrane structure associated with the multifocal synapse and T-cell clustering. *Blood 112:* 5037-5045
24. FLAMAND V, SORNASSE T, THIELEMANS K, DEMANET C, BAKKUS M, BAZIN H, TIELEMANS F, LEO O, URBAIN J, MOSER M 1994 Murine dendritic cells pulsed in vitro with tumor antigen induce tumor resistance *in vivo*. *Eur J Immunol 24:* 605-610
25. FOX B A, SCHENDEL D J, BUTTERFIELD L H H *et al.* 2011 Defining the critical hurdles in cancer immunotherapy. *J Transl Med 9:* 214
26. FULOP T, LARBI A, KOTB R, DE ANGELIS F, PAWELEC G 2011 Aging, immunity, and cancer. *Discovery Med 11:* 537-550
27. GALON J, COSTES A, SANCHEZ-CABO F, KIRILOVSKY A, MLECNIK B, LAGORCE-PAGÈS C, TOSOLINI M, CAMUS M, BERGER A, WIND P, ZINZINDOHOUÉ F, BRUNEVAL P, CUGNENC P-H, TRAJANOSKI Z, FRIDMAN W-H, PAGÈS F 2006 Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science 313:* 1960-1964
28. GENTLES A J, GALLAHAN D 2011 Systems biology: Confronting the complexity of cancer. *Cancer Res 71:* 5961-5964
29. GOMELLA L, NABHAN C, DEVRIES T, WHITMORE J, FROHLICH M, GEORGE D 2012 Estimating the overall survival benefit of sipuleucel-T in the IMPACT trial accounting for crossover treatment in control subjects with autologous immunotherapy generated from cyropreserved cells. American Urological Association Annual Meeting, Abstract No. 683.
30. ROBINS J M, TSIATIS A A 1991 Correcting for non-compliance in randomized trials using rank preserving structural failure time models. *Commun Statist Theory Meth 20:* 2609–2631
31. HELLSTROM I, HELLSTROM K E, PIERCE G E, YANG J P 1968 Cellular and humoral immunity to different types of human neoplasms. *Nature 220:* 1352-1354
32. HIGANO C S, SCHELLHAMMER P F, SMALL E J, BURCH P A, NEMUNAITIS J J, YUH L, PROVOST N, FROLICH M W 2009 Integrated data from 2 randomized double-blind, placebo--controlled, phase 3 trials of active cellular immunotherapy with sipuleucel-T in advanced prostate cancer. *Cancer 115:* 3670-3679
33. HOLTAN S G, DRONCA R S, NEVALA W K, PORRATA L F, MANSFIELD A S, BOLOCK M S, LEONTOVICH A A, GROTZ T E, TURNER J D, FRISCH H P, MARKOVIC S N 2011 The dynamic human immune response to cancer: It might just be rocket science. *Immunotherapy 3:* 1021-1024
34. HOOS A, BRITTEN C M, HUBER C, O'DONNELL-TORMEY J 2011 A methodological framework to enhance the clinical success of cancer immunotherapy. *Nature Biotechnol 29:* 867-870
35. HOPTION CANN S A, VAN NETTEN J P, VAN NETTEN C, GLOVER D W 2002 Spontaneous regression: A hidden treasure buried in time. *Med Hypotheses 58:* 115-119
36. HRUSHESKY W J 1985 Circadian timing of cancer therapy. *Science 228:* 73-75
37. HSU F J, BENIKE C, FAGNONI F, LILES T M, CZERWINSKI D, TAIDI B, ENGLEMAN EG, LEVY R 1996 Vaccination of patients with B-cell lymphoma using autologous antigen-pulsed dendritic cells. *Nature Med 2:* 52-58
38. HUBER M L, HAYNES L, PARKER C, IVERSEN P 2012 Interdisciplinary critique of sipuleucel-T as immunotherapy in castration-resistant prostate cancer. *J Natl Cancer Inst 104*: 273-279
39. IANKOV I D, BLECHACZ B, LIU C, SCHMECKPEPER J D, TARARA J E, FEDERSPIEL M J, CAPLICE N, RUSSELL S J 2007 Infected cell carriers: A new strategy for systemic delivery of oncolytic measles viruses in cancer virotherapy. *Mol Therapy 15:* 114-122
40. ISHII K, KURITA-TANIGUCHI M, AOKI M, KIMURA T, KASHIWAZAKI Y, MATSUMOTO M, SEYA T 2005 Gene-inducing program of human dendritic cells in response to BCG cell-wall skeleton (CWS), which reflects adjuvancy required for tumor immunotherapy. *Immunol Letters 98:* 280–290
41. KANTOFF P W, HIGANO C S, SHORE N D, BERGER E R, SMALL E J, PENSON D F, REDFERN C H, FERRARI A C, DREICER R, SIMS R B, XU Y, FROLICH M W, SCHELLHAMMER PF 2010 Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *New Engl J Med 363:* 411-422
42. KAUFFMAN S A 2006 Beyond reductionism: Reinventing the sacred. http://www.edge.org/3rd_culture/kauffman06/kauffman06_index.html.
43. KIRSCHNER D, PANETA J C 1998 Modeling immunotherapy of the tumor-immune interaction. *J Math Biol 37:* 235-252
44. KOGAN Y, HALEVI-TOBIAS K, ELISHMERENI M, VUK-PAVLOVIĆ S, AGUR Z 2012 Reconsidering the paradigm of cancer

immunotherapy by computationally aided real-time personalization. *Cancer Res 72:* 2218-2227

45. KRONIK N, KOGAN Y, ELISHMERENI M, HALEVI-TOBIAS K, VUK-PAVLOVIĆ S, AGUR Z 2010 Predicting outcomes of prostate cancer immunotherapy by personalized mathematical models. *PLoS ONE 5:* e15482
46. LACY M Q, MANDREKAR S, DISPENZIERI A, HAYMAN S, KUMAR S, BUADI F, DINGLI D, LITZOW M, WETTSTEIN P, PADLEY D J, KABAT B, GASTINEAU D, RAJKUMAR S V, GERTZ M A 2009 Idiotype-pulsed antigen presenting cells following autologous transplantation for multiple myeloma may be associated with prolonged survival. *Am J Hematol 84:* 799-802
47. LEONTOVICH A A, DRONCA R S, SUMAN V J, ASHDOWN M L, NEVALA W K, THOMPSON M A, ROBINSON A, KOTTSCHADE L A, KAUR J S, MCWILLIAMS R R, IVANOV L V, CROGHAN G A, MARKOVIC S N 2012 Fluctuation of systemic immunity in melanoma and implications for timing of therapy. *Front Biosci 4:* 958-975
48. MARINCOLA F M, JAFFEE E M, HICKLIN D J, FERRONE S 2000 Escape of human solid tumors from T-cell recognition: Molecular mechanisms and functional significance. *Adv Immunol 74:* 181-273
49. MEHTA-DAMANI A, MARKOWITZ S, ENGLEMAN EG 1994 Generation of antigen-specific $CD8^+$ CTLs from naive precursors. *J Immunol 153:* 996-1003
50. MELLMAN I, NUSSENZWEIG M 2011 Ralph M. Steinman (1943-2011). *Science 334:* 466.
51. MICHAEL A, BALL G, QUATAN N, WUSHISHI F, RUSSELL N, WHELAN J, CHAKRABORTY P, LEADER D, WHELAN M, PANDHA H 2005 Delayed disease progression after allogeneic cell vaccination in hormone-resistant prostate cancer and correlation with immunologic variables. *Clin Cancer Res 11:* 4469-4478
52. MLECNIK B, TOSOLINI M, KIRILOVSKY A, BERGER A, BINDEA G, MEATCHI T, BRUNEVAL P, TRAJANOSKI Z, FRIDMAN W-H, PAGÈS F, GALON J 2011 Histopathologic-based prognostic factors of colorectal cancers are associated with the state of the local immune reaction. *J Clin Oncol 29:* 610-618
53. NORTH R J, AWWAD M 1990 Elimination of cycling $CD4^+$ suppressor T cells with anti-mitotic drug releases non-cycling $CD8^+$ T cells to cause regression of an advanced lymphoma. *Immunology 71:* 90-95
54. PAGANI P, PEPPONI R, FUGGETTA M P, PRETE S P, TURRIZIANI M, BONMASSAR L, LACAL P M, FALCINELLI S, PASSARELLI F, GUADAGNI F, ALVINO E, D'ATRI S 2003 DNA repair enzymes and cytotoxic effects of temozolomide: Comparative studies between tumor cells and normal cells of the immune system. *J Chemotherapy 15:* 173-183
55. PAYNE H, CORNFORD P 2011 Prostate-specific antigen: An evolving role in diagnosis, monitoring, and treatment evaluation in prostate cancer. *Urol Oncol 29:* 593-601
56. RYAN E M, GORMAN S P, DONNELLY R F, GILMORE B F 2011 Recent advances in bacteriophage therapy: How delivery routes, formulation, concentration and timing influence the success of phage therapy. *J Pharmacy Pharmacol 63:* 1253-1264
57. SAVA G, GIRALDI T, TOMAŠIĆ J, HRŠAK I 1983 Immunotherapy of Lewis lung carcinoma with hydrosoluble peptidoglycan monomer (PGM). *Cancer Immunol Immunother 15:* 84-86
58. SHEIKH N A, JONES L A 2008 CD54 is a surrogate marker of antigen presenting cell activation. *Cancer Immunol Immunother 57:* 1381-1390
59. SMALL E J, FRATESI P, REESE D M, STRANG G, LAUS R, PESHWA M V, VALONE F H 2000 Immunotherapy of hormone-refractory prostate cancer with antigen-loaded dendritic cells. *J Clin Oncol 18:* 3894-3903
60. SMALL E J, SCHELLHAMMER P F, HIGANO C S, REDFERN C H, NEMUNAITIS J J, VALONE F H, VERJEE S S, JONES L A, HERSHBERG R M 2006 Placebo-controlled phase III trial of immunologic therapy with sipuleucel-T (APC8015) in patients with metastatic, asymptomatic hormone refractory prostate cancer. *J Clin Oncol 24:* 3089-3094
61. STEINMAN R M, COHN Z A 1973 Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *J Exp Med 137:* 1142-1162
62. STREBHARDT K, ULLRICH A 2008 Paul Ehrlich's magic bullet concept: 100 years of progress. *Nature Rev Cancer 8:* 473-480
63. SYLVESTER R J 2011 Bacillus Calmette-Guerin treatment of non-muscle invasive bladder cancer. *Int J Urol 18:* 113-120
64. THOMAS L 1969 Discussion. *In:* Lawrence H S *(ed)* Cellular and Humoral Aspects of the Hypersensitive States: A Symposium at the New York Academy of Medicine. Paul B. Hoeber, Inc., New York, p 530
65. TIMMERMAN J M, CZERWINSKI D K, DAVIS T A, HSU F J, BENIKE C, HAO Z M, TAIDI B, RAJAPAKSA R, CASPAR C B, OKADA C Y, VAN BECKHOVEN A, LILES T M, ENGLEMAN E G, LEVY R 2002 Idiotype-pulsed dendritic cell vaccination for B-cell lymphoma: Clinical and immune responses in 35 patients. *Blood 99:* 1517-1526
66. Tishler M, Shoenfeld Y 2006 Bcg immunotherapy–from pathophysiology to clinical practice. *Expert Opin Drug Safety 5:* 225-229
67. Tsung K, Norton J A 2006 Lessons from Coley's toxin. *Surg Oncol 15:* 25-28
68. VAN DER BRUGGEN P, ZHANG Y, CHAUX P, STROOBANT V, PANICHELLI C, SCHULTZ ES, CHAPIRO J, VAN DEN EYNDE B J, BRASSEUR F, BOON T 2002 Tumor-specific shared antigenic peptides recognized by human T cells. *Immunol Rev 188:* 51-64
69. VUK-PAVLOVIĆ S 2008 Rebuilding immunity in cancer patients. *Blood Cells Mol Disease 40:* 90-100