« New Prospects for Cancer Immunotherapy: Integrating Biomarkers in Mathematical Models » Kibbutz Dalia, Israel, May, 4-6, 2008

Abstracts

A new breast cancer therapy using Dickkopf (dkk) protein for diverting proliferating cancer stem cells to terminal differentiation: evaluation by a mathematical model

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Breast cancer is the most prevalent oncological disease among women worldwide, current therapies still falling short of providing effective remission and diminished risk of recurrence. Recent studies, implicating the involvement of breast cancer stem cells (CSCs) in sustaining tumor growth and recurrence, and the finding that these cells can escape conventional therapy, underline the difficulty in curing the disease. However, novel therapy avenues can be uncovered by deciphering the molecular mechanisms governing mammary CSCs fate decision, and by understanding how to divert proliferating cells into differentiation. Mammary stem cells are controlled by complex inter-relationships between several pathways, notably Wnt and Notch. The Dkk protein family is a target of the Wnt signaling pathway and several members of this family negatively regulate this pathway's activity. Cross-talking with the Wnt signaling pathway is the Notch signaling pathway, which is regulated by the Delta, Serrate, Lag-2 (DSL) ligand.

Our aim in this work is to study the complex interactions involved in breast CSC proliferation, and to identify the crucial factor, whose modulation can redirect these cells into terminal differentiation. We do so by mathematically analyzing the involved dynamics, which may be intractable to experimental investigation. Our mathematical model is a system of 7 differential equations, describing the major signaling pathways governing SC development and their interactions. Using this model we numerically simulated the effects of in situ Dkk administration on the normal SC proliferation and on the proliferation of mammary CSCs.

Results suggest a biphasic effect of Dkk on breast CSCs. Low Dkk levels accelerate mammary stem cell proliferation, whereas high Dkk levels drive them into differentiation, ultimately leading to tumor elimination. Moreover, our work indicates that mutations in the Wnt and the E-cadherin pathways, but most significantly in the Notch pathway, result in increased CSC proliferation, as compared to the wild-type stem cells. Furthermore, mutations in the Wnt pathway, but not in the Notch pathway, result in moderately increased Dkk secretion. The increased intercellular Dkk levels can suffice for arresting the proliferation of wild type stem cells but not of the mutated cells.

These results imply that breast cancer metastysing cells, which bear additional mutations in the Wnt pathway, and hence over-express Dkk, can annihilate surrounding wild-type stem cells while preserving their own augmented proliferation. Taken together our findings suggest that a continuous treatment with high Dkk doses can promote CSC differentiation and drive the cancerous tissue to exhaustion.

Validation of this suggestion in preclinical and clinical research will clarify the efficacy of this new therapy strategy.

The role of T cells and anti-3rd party autologous cytotoxic T cells (CTLs) in tumor progression and the eradication of B chronic lymphocytic leukemia.

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The severe immunological imbalance, characterizing B CLL during progression of disease is most probably related by total number of T cells expressed by CD3. In parallel the expression of CD5 (also a T cell marker) is more pronounced. In a study of 625 patients we found statistically significant correlation (univariate) between disease severity (both Rai and Binet score) and expression of CD3. We also found a significant inverse correlation between CD3 and CD5. We therefore investigated the expression of T cell associated antigen markers as expressed the ratio CD5-CD3/CD3. The ratio was significantly associated with disease severity both in a univariate mode ($p<0.005$) and in a multivariate regression model ($p<0.001$), after adjusting for gender and disease type (typical and atypical). The ratio represents a mathematical expression of the inverse correlation between CD3 and CD5. We believe that the implication of the correlation between high to low CD5-CD3/CD3 ratio and disease severity may be used as a sensitive prognostic factor for clinical practice in the management of CLL.
B CLL cells might be regulated by inhibitory and/ or growth promoting signals exerted by autologous T cells. Using chimeric SCID mouse model we showed that transplantation of PBL cells from B CLL patients at low stages of disease leads to marked engraftment of T cells, whereas infusion of PBLs from patients in advanced stages leads to predominant engraftment of malignant B cells. These results supported our working hypothesis that autologous T cells can actively suppress the expansion of the CLL in our animal model. Therefore we attempted to expand T cell purified from advanced B CLL patients by stimulating them against 3rd party stimulator (EBV-transformed line and later IL-2). We found that these autologous T cells not only can be considerably expanded but they also acquired a potent GVL effect in vivo showing a marked leukemia eradication (up to 80%) in the B CLL transplanted into the human-mouse chimeric model. This experiment was performed in 6 stage 4 B CLL patients. In vitro studies allowed us to investigate the mechanism of autologous anti-3rd party CTLs. We found that cell contact induced the expression of the adhesion molecule ICAM-1 (CD54) and enhanced apoptosis of B cells together with expression of fas (CD95). Transwell system avoiding contact between CTLs and B CLL cells and the use anti- LFA-1 antibodies inhibited this phenomenon.

In conclusion, our wide statistical analysis emphasized the inverse correlation between T cells and disease severity in B CLL. Autologous anti-3rd party cytotoxic T cells expanded from stage 4 CLL patients are endowed with the potent eradication effect of B CLL transplanted into animals suggesting a rational for preserving and expanding autologous T cells for the management of progressing B CLL.

Antigen microarray chip, autoantibody profiles and immune biomarkers

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The immune system contains a representation of key body molecules encoded in its repertoire of autoantibodies binding to these molecules – what I have called the immunological homunculus; the immune system's representation of the body. To characterize this homunculus, we have used informatic tools to study patterns of antibodies to many hundreds of self-molecules arrayed on glass slides – an antigen chip of our design. Results using the antigen chip suggest that the particular self-reactivities comprising the homunculus could serve as a set of biomarkers that help the immune system initiate and regulate the inflammatory processes that maintain the body. These immune profile biomarkers can also be used for biomedical ends.

Cancer immunotherapy by interleukin-21: developing novel treatment strategies by a validated mathematical pharmacokinetic & pharmacodynamic model

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The newly characterized interleukin (IL)-21 plays a central role in the transition from innate immunity to adaptive immunity and shows anticancerous effects in mice. IL-21 is now developed as an immunotherapeutic drug for malignant diseases, but conditions for efficacious therapy, and the conflicting immunostimulatory and immunoinhibitory influences of the cytokine, are yet to be defined. We studied the effects of IL-21 on tumor eradication in a mathematical model focusing on natural killer (NK) cell- and CD8+ T-cell-mediated lysis of tumor cells. Model parameters were estimated using a variety of studies, in which mice with non-immunogenic melanoma and immunogenic fibrosarcoma were treated with IL-21 via cytokine gene therapy. With an additional pharmacokinetic component, the model's accuracy was validated by data from retrospective preclinical research in melanoma and renal-cell carcinoma-bearing mice that received standard subcutaneous or intraperitoneal injections of IL-21. The model emphasizes the importance of tumor immunogenicity to the IL-21-mediated anticancerous response, showing a strong dependence of the NK-cell/CD8+ T-cell balance on this characteristic. While simulations of "cytokine-gene therapy"-like regimens dynamically determined according to tumor mass changes result in efficient elimination of non-immunogenic melanoma, in immunogenic tumors this strategy was not found to be superior to that of "fixed" standard dosing regimens. Using a customized optimization method considering therapeutic efficacy and drug-associated toxicity, the model also predicts that in melanoma, substantial cancer regression can be achieved with very low amounts of IL-21 applied with non-uniform inter-dosing intervals. Collectively, our model supports clinical use of IL-21 as a potent stimulator of cellular immunity against cancer, yet suggests selecting the therapeutic strategy according to tumor immunogenicity and mass, as well as drug toxicity considerations.

A new strategy for improving interleukin-11 therapy for thrombocytopenia based on a mathematical pharmacokinetic/pharmacodynamic analysis

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Interleukin (IL)-11, a pleiotropic growth factor with biological effects on a variety of cell types, has a central role in stimulating megakaryocytopoiesis and platelet production. In human trials, this cytokine has been shown to alleviate chemotherapy-induced thrombocytopenia in patients with solid tumors. Yet standard IL-11 treatment (50-75 μg/kg/day doses) is accompanied by a highly complex toxicity profile, significantly limiting its clinical use. Our aim was to develop improved therapeutic strategies for IL-11, by mathematically analyzing the beneficial and detrimental effects of this drug. An ordinary differential equation pharmacokinetic (PK)/ pharmacodynamic (PD) model for IL-11-therapy was developed and computerized. The model considered thrombopoietic effects of the cytokine, together with the increase in acute-phase inflammatory proteins. Model parameters were estimated using human data of IL-11 PK/PD and applying customized local and global search heuristics. Therapeutic regimens achieving maximal platelet elevation (efficacy) together with minimal inflammation (toxicity) were explored. The PK/PD model, with alternative parameter sets, satisfyingly retrieved platelet dynamics and inflammatory effects following IL-11 therapy, and showed robust behavior even beyond the therapeutic time window. Higher efficacy/toxicity ratios were predicted to be achieved under a therapeutic schedule consisting of 5-10 μg/kg/day doses, a 2-3 day inter-dosing interval, and a 4-week or more therapeutic window. A more efficacious treatment was predicted for regimens composed of different doses for every day of therapy. We present here, for the first time, a PK/PD mathematical model of IL-11 supportive treatment for chemotherapy-associated toxicities, which suggests clinically applicable improved treatment schedules of lower intensity and longer duration than currently recommended regimens. These may allow more widespread application of IL-11 in clinic, and reduce the need for transfusion of platelets.

IL-21: a pleiotropic cytokine and a new tool cancer immunotherapy

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IL-21 is a recently discovered class I cytokine, which is produced by activated Th2, Th17 and NK-T cells. IL-21 biological activities are mediated by a specific IL-21R, structurally related to the IL-2Rbeta chain, which associates with the common gamma chain for signal transduction. IL-21 co-stimulates the proliferation of T and NK lymphoid cells and potentiates their cytolytic functions. In addition, it regulates B cell proliferation and survival and induces B cell differentiation and IgG1 production. Besides its activities on normal lymphoid cells, IL-21 is an in vitro growth factor for myeloma and acute-T cell leukemia cells, whereas it induces the apoptosis of B-CLL (chronic lymphocytic leukemia) cells. By contrast the structurally related cytokine IL-15 induces B-CLL cell proliferation and survival. These opposite effects are related to the activation of different signal transduction pathways by IL-21 (STAT1 and STAT3) and IL-15 (STAT5 and ERK1/2). These data suggest that IL-21 administration or pharmacological blockade of IL-15 signaling pathways may be further explored in B-CLL therapy.

Differently from IL-2, IL-21 is not specifically required for the development and the proliferation of regulatory T (Treg) cells and does not support their immune-suppressive functions, suggesting that it may represent a new tool for cancer immunotherapy. Indeed, IL-21 has shown anti-tumour activity in a variety of murine experimental tumour models due to its ability to activate specific or innate immune responses against neoplastic cells. The induction of tumour-specific immunity by IL-21 may involve either CTL responses or antibody production, depending on the experimental tumour model considered. For example, we found that rIL-2 or IL-2-gene modified tumor cell vaccines cure a fraction of syngneic mice bearing mammary adenocarcinoma micrometastases, through CTL- and IFN-gamma-dependent mechanisms. These effects could be potentiated by co-administration of an anti-CD25 mAb, which blocks Treg cell functions. Differently, the stereotactic delivery of rIL-21 or of IL-21-transduced cells into orthotopically implanted gliomas cured most of glioma-bearing mice through the induction of a protective antibody response. IL-21 may also increase the therapeutic activity of antibodies targeting tumour cells, through the enhancement of ADCC (antibody-dependent cellular cytotoxicity) mediated by NK cells, providing the rationale for IL-21-based therapeutic combinations. Recently, IL-21 has entered phase-I clinical studies, which showed that the use of IL-21 is feasible and may result in immune-enhancing effects in melanoma and renal cancer patients.

Improving alloreactive CTL immunotherapy for malignant gliomas using a simulation model of their interactive dynamics– identifying efficacy biomarkers

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Glioblastoma (GBM), a highly aggressive (WHO grade IV) primary brain tumor, is refractory to traditional treatments, such as surgery, radiation or chemotherapy. This study aims at aiding in the design of more efficacious GBM therapies. We constructed a mathematical model for glioma and the immune system interactions, that may ensue upon direct intra-tumoral administration of ex vivo activated alloreactive cytotoxic-T-lymphocytes (aCTL). Our model encompasses considerations of the interactive dynamics of aCTL, tumor cells, major histocompatibility complex (MHC) class I and MHC class II molecules, as well as cytokines, such as TGF-β and IFN-γ, which dampen or increase the pro-inflammatory environment, respectively. Computer simulations were used for model verification and for retrieving putative treatment scenarios. The mathematical model successfully retrieved clinical trial results of efficacious aCTL immunotherapy for recurrent anaplastic oligodendroglioma and anaplastic astrocytoma (WHO grade III). It predicted that cellular adoptive immunotherapy failed in GBM because the administered dose was 20-fold lower than required for therapeutic efficacy. Model analysis suggests that GBM may be eradicated by new dose-intensive strategies, e.g., 3×108 aCTL every 4 days for small tumor burden, or 2×109 aCTL, infused every 5 days for larger tumor burden. Further analysis pinpoints crucial bio-markers relating to tumor growth rate, tumor size, and tumor sensitivity to the immune system, whose estimation enables regimen personalization. Further mathematical analysis improved our mathematical model through cancellation of negligible terms and proved the existence of an effective cellular immunotherapy within the range of the physiological parameters we use. We propose that adoptive cellular immunotherapy was prematurely abandoned. It

may prove efficacious for GBM, if dose intensity is augmented, as prescribed by the mathematical model. Re-initiation of clinical trials, using calculated individualized regimens for grade III–IV malignant glioma, is suggested.

Integrated bone homeostasis model for denosumab pharmacodynamics in multiple myeloma

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There is considerable interest in utilizing cell systems models to interpret the time-course of clinical biomarkers of drug effects. This paper will describe efforts to integrate a cellular bone homeostasis model with the pharmacokinetics (PK) and mechanism of action of denosumab, an inhibitor of receptor activator of nuclear factor-κB ligand, to characterize the time course of serum N-telopeptide (NTX), a bone resorption biomarker, following single escalating doses in multiple myeloma (MM) patients. Mean PK and median serum NTX temporal profiles were extracted from the literature. Nonlinear denosumab PK profiles were well described by a model that includes rapid binding of the drug to its pharmacological target. PK profiles were integrated into a previously reported theoretical cellular model of osteoblast-osteoclast interactions. Serum NTX concentrations were linked to a resorbing active osteoclast (AOC) pool by a nonlinear transfer function. Reasonable fits were obtained for the NTX profiles from maximum likelihood estimation using the final model. Transfer function parameters, including the basal NTX level independent of AOC and the AOC concentration producing 50% of maximal NTX production, were estimated with good precision as 5.7nM and 1.2x10-5pM. In summary, an integrated cellular model was developed to quantitatively describe clinical biomarker data and may provide a means for testing hypotheses on the role of cellular system components in controlling drug response.

Technologies for local drug delivery into the brain - the concept of convection-enhanced delivery

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Getting drugs into the brain is a challenge due to the effective physiological function of the blood brain-barrier (BBB). Accordingly, drugs that may be effective *in vitro* against a variety of brain pathologies, including neoplastic, degenerative and metabolic disorders, have no clinical relevance when administered systemically.
Various attempts have been made in the past to bypass the BBB by direct injection into the brain, using the CSF as a conduit, intra-arterial infusions of drugs, diffusion-based technologies, as well as other approaches that have uniformly failed to achieve an effective drug concentration in the brain, or selected targets within.

Convection-enhanced drug delivery (CED) is a novel technique where small volumes of a therapeutic drug are slowly infused into the brain over a long period of time. This technique has been shown to enable reaching drug concentrations that are orders of magnitude higher than those attainable with systemic delivery. As a platform technology, CED can be used to treat brain tumors, deliver drugs to degenerative

brain conditions (such as Parkinson's disease and Alzheimer), as well as other brain pathologies.
This technology, although seemingly simple, is in fact a very complicated process that is dependent on a large number of variables, including physical and chemical characteristics of the infusate, its polarity, hydrophilicity, molecular weight, to mention but a few.

Monitoring the convective process in a real-time fashion is another complicated problem. Experience from clinical studies have shown that although convection can be modeled using mathematical equations, the actual effect is almost always different than the expected outcome. This is the result of variable clearance of the infusate, leakage through paths of low resistance, *in vivo* degradation and physical barriers.

We will present our experience from clinical studies using convection for the treatment of brain tumors and our development program for optimizing convection, enhancing the target volume of distribution, and means to monitor convection and predict tumor response to treatment.

From bone marrow to HLA-disparate stem cell grafts - a platform for cellular immunotherapy

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Allogeneic bone marrow or stem cell transplantation is the only curative approach for a variety of malignant as well as non-malignant diseases. The use of bone marrow as allogeneic stem cell source has been continuously replaced by peripheral blood stem cells. Characterization of stem cell markers such as CD34, CD133 and others has enabled to select hematopoietic progenitor stem cells from distinct immunological effector cells within the graft. The development of CD34 selection has helped to establish haploidentical stem cell transplantation from a parental HLA-mismatched donor for a child as the treatment of choice for situations where an actual HLA-identical unrelated donor is not available. CD34 selection has enabled the transplantation and successful engraftment of stem cells across major HLA-barriers. Our group has performed extensive research on the characterization of the distinct phases of immune reconstitution, the factors that govern the onset and plateau of thymic function as well as CDR3-spectratyping of T cell subpopulations following transplantation of highly purified CD34 selected stem cell grafts. Several recent developments have refined this technique in the pediatric transplant setting: CD3/CD19 depletion allows for more committed progenitor cells especially lymphocyte progenitors to be included in the graft without the risk of increased GVHD. Preliminary data indicate that immune reconstitution is faster than with CD34 selected grafts. These novel transplantation approaches for children with malignant diseases at very high risk of relapse offer a cellular platform for later use of distinct cellular effector cell populations with specificity for tumor antigens (WT-1) or other antigens of interest.

Physiologically-based pharmacokinetics & pharmacodynamics model of targeted drug delivery by monoclonal antibodies – searching for personal biomarkers

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For the last few decades, modern pharmacology, in general, and hematological cancers, in particular, largely benefited from the development of molecular targeted drugs on the basis of monoclonal antibodies (MA). Cancer cells can develop resistance to the conjugated antibodies by several mechanisms such as low expression of the target membrane antigen, rapid metabolism, rapid excretion from the cell, or resistance to the conjugate toxin. All the aforementioned resistance mechanisms contribute to the high response variability already seen in MA-based drugs (MA-BD). We present a physiologically-based MA-BD PK/PD mathematical model that includes blood PK and detailed model of MA-BD interactions with its target receptor. It can be coupled with various indirect response mechanism-based PD. We applied our model to experimental data of Gemtuzumab Ozogamycin interaction with leukemic blasts in vitro and in vivo in order to evaluate individual model parameter values in the patient population. Mathematical analysis of model behavior under physiological parameter value ranges allowed for formulation of general principles of treatment by targeted drug delivery, including identification of parameters with highest influence on drug efficacy and optimization of treatment schedule.

Markers, Mathematics, Immunotherapy and Malignancy - a Clinician's Perspective

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Circulating biologic markers in blood have been playing an increasingly important role in clinical medicine, but markers mean different things to different investigators under different circumstances.
There are useful makers for diagnosis or identifying disease recurrance, for assessing prognosis, and for assessing effect of therapy. Unfortunately problems of sensitivity and specificity limit the number of established makers to only a few disease categories and conditions. The uncritical use of markers can be misleading however and the FDA and oncological societies have been very reflective before recommending the use of any particular marker. For monitoring immune therapy, reliable markers are urgently needed. The practice of sampling blood for measurement of antibody levels or cell-mediated immunity may provide data correlated , even very strongly, to tumor regression but such correlation is not sufficient for establishing the candidate assay as a reliable marker . While lack of standardization between laboratories is frequently cited as a key problem, there are major serious inherent problems that have to date, kept the goal of identifying surrogate markers so far out of reach. One recent validated model (de Pellis et al, 2005) shows that both NK and CD8+ cells are needed for an effective immune response in a coordinated process governed by complex dynamics. Measurement of, say levels of cytotoxic CD8+ cells in the peripheral blood at some arbitrary time after therapy may or may not be be a relevant surrogate for immunologic cell kill at the site of tumor and hence subsequent clinical regression. The discussion will consider cytotoxic cells as a pathogen for tumors, and review Koch's postulates as a template for defining the necessary and sufficient

conditions for identifying immune markers relevant and informative in immunotherapy clinical trials.

Tumor-specific immunotherapy for high grade glioma using ex vivo expanded autologous T-cells: therapeutic potential or utopia?

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Patients with high grade glioma (HGG) have a median life expectancy of 13-15 months after diagnosis, despite all therapeutic efforts combining surgery, chemotherapy and radiation. Recurrence at the original tumor site is the common pattern of relapse, and the extent of gross total surgery is the factor which inversely correlates with the risk of relapse . This emphasizes the role of residual tumor cells in the tumor bed and calls for more efficient therapeutic approaches to reduce these residual tumor cells to a minimum.

Immunotherapy is an additional therapeutic option for HGG-patients, however, the critical hurdles are how to obtain T-cells fast, in order to provide sufficient numbers to eradicate the growing tumor cells, how to get the T-cells to the tumor site – as homing across the blood-brain-barrier is required, and how to induce fully functional T-cells to resist the inhibitory tumor-micromilieu. Transfer of ex vivo expanded, tumor-specific T-cells may overcome these hurdles, as the cells 1) can be expanded to very high numbers, 2) can then be injected directly into the tumor cavity, thus bypassing the BBB and 3). can be manipulated ex vivo to induce resistance against tumor-derived inhibitory factors to have increased lytic capacity.

We have developed methods to induce and expand a tumor-specific T-cell response in vitro even against antigens, against which no pre-formed immunity existed. By combining novel culture techniques like the use of IL-21 and a selection strategy based on the expression of CD137 upon activation of the T-cells, it is possible to generate and study purified T-cell populations against glioma-associated antigens like survivin, IL-13-receptor-alpha-2 and EphA2. We will present strategies how to improve the efficacy of these T-cells so that they can overcome the inhibitory environment generated by the glioma cells. Furthermore the requirements for clinical application, which include the production according to good manufacturing practice (GMP) as well as the potential advantage of mathematical modelling – from a physician´s point of view - will be discussed.