PREFACE

Though the clinician usually encounters a malignant tumor in its progression phase, the reverse process of permanent spontaneous regression or remission of malignant tumors is a well-documented phenomenon, occurring subclinically across human populations, for instance at 22–46% rate in breast cancer, as per the Scandinavian and Wisconsin Screening Registries which have tracked a population of 0.33 million and 2.95 million individuals respectively. Complete spontaneous tumor regression (without treatment) is well observed to occur in animals and humans as epidemiological analysis show, whereby the malignancy is permanently eliminated without any treatment. As per PubMed, there are about 14,000 titles of papers dealing with spontaneous cancer regression, covering virtually all types of malignant diseases such as sarcomas, carcinomas, lymphomas, melanomas and so on. Though the regression process eliminates malignant cells, it does not damage the normal tissue, i.e. normal cells are protected overall. Spontaneous cancer regression depends on various factors like the amount of the tumor load, the invasiveness of the disease, the intensity of the treatment, and the robustness of the patient's immune response. Mathematical modelling is acknowledged to be a seminal tool for creating better understanding of tumour behaviour as well as to develop novel treatment strategies for cancer patients.

There are several significant drawbacks to cancer treatment by antitumor agents, such as chemotherapy and immunotherapy. The first issue is an apparent “clinical cure” whereby the tumor cells are eliminated to a major extent, so that the tumor is clinically undetectable, even though microscopic amounts of cancer cells remain, which flare up much later after the initial therapy, thus producing tumor recurrence. The second is presence of cancer stem cells which, even though initially forming a miniscule cellular population, goes on multiplying as they have much less sensitivity to therapeutic agents,

thus producing a resistant tumor relapse. Another issue is the inability of administering therapeutic agents intensively, as the latter produces appreciable normal tissue damage, producing hazardous damaging side-effects that prevent the administration of further therapy. These disadvantages need to be well address, though it is known that sometimes there is permanent elimination of a tumor by therapeutic agents. Such therapy uses multimodal combined chemotherapy and immunotherapy drugs (like alkylators as dacarbazine or temozolomide), antitumor lymphocyte therapy, along with interleukin-2.

In the first phase of our work, the tumor system behavior has been investigated by taking melanoma as a case study. Firstly, the conventional protocol of chemotherapy and immunotherapy of melanoma was formulated. In this case the tumor cell population initially decreases to about 1% of the initial malignant cells, but later after the therapy duration is over, these residual cancer cell increases to high values (≈109 malignant cells) there is a tumour relapse, such a tumor recurrence will then penetrate the blood vessels, producing wide dissemination and lethality.

To counter this issue, we formulated the use of negative biasing technique as a novel computational systems biology procedure to enable full extension of all the tumour cells (including cancer stem cell) and enable complete permanent regression of the tumour. Thereby we obtain incisive insights into the possibility of therapeutically replicating such complete regression processes on tumors clinically, without toxic side effects. Oncological informatics approach using cell-kinetics based coupled differential equations was formulated while protecting normal tissue. Here, the three main anti-tumour entities were investigated: (i) DNA blockade factor, (ii) Interleukin-2 (IL-2), and (iii) Cytotoxic T-cells (CD8+ T).

In the next phase of the thesis, the temporal variations of the above mentioned three entities were mathematically formulated and then investigated from a cancer biology basis,

utilizing preclinical experimental investigations on malignant tumors, using mammalian melanoma microarray and histiocytoma immunochemical assessment. The study found that permanent tumor regression can occur by:

(1) Negative-Bias shift in population trajectory of tumor cells, eradicating them under first-order asymptotic kinetics, and

(2) Temporal alteration in the three antitumor entities (DNA replication-blockade factor, Antitumor T-lymphocyte, IL-2), these three entities being respectively characterized by the following temporal pattern in the variation of their blood concentration namely: (a) Unimodal Inverted-U function, (b) Bimodal M-function, (c) Stationary-step function.

These three patterns provide a time-wise orchestrated tri-phasic anti-tumour profile for eradicating the tumour. The study also elucidated gene-expression levels corresponding to the above three components: (i) DNA-damage G2/M checkpoint regulation [genes: CDC2, CHEK], (ii) Chemokine signaling: IL-2/15 [genes: IL2RG, IKT3], (iii) T-lymphocyte signaling (genes: TRGV5, CD28). The temperature variation of all the three gene expression levels quantitatively followed the same activation profiles predicted by our computational model (Smirnov-Kolmogorov statistical test satisfied, $\alpha = 5\%$). Furthermore, we have shown that the genes CASP7 and GZMB are signatures of the Negative-bias dynamics, enabling extinction of the residual tumor. Using the negative-biasing principle, the investigation also furnished the dose-time profile of equivalent therapeutic agents (DNA-alkylator drug, IL-2, T-cell input) so that the tumor may be made to therapeutically undergo permanent eradication by replicating the spontaneous tumor regression dynamics.

In the next phase of the thesis, we developed a systems biological formulation of the regression process with validation by experimental finding and identified the relevant candidate biomolecules for therapeutic utility. As a case study, we analyzed the time-wise

biopsy and microarray of spontaneously regressing melanoma and immunochemical analysis of spontaneously regressing fibrosarcoma tumors in mammalian/human hosts. Then, we analyzed the differentially-expressed genes (DEGs), signaling pathways, and bioinformatics framework of regression. Additionally, prospective biomolecules that could induce a complete tumor regression are investigated. We identified 176 upregulated and 116 downregulated DEG genes, and enrichment analysis showed that the most significant ones were downregulated cell-division genes: TOP2A, KIF20A, KIF23, CDK1 and CCNB1. Moreover, enrichment analysis also shows that the most influential genes for complete tumour eradication are those whose downregulation produces arrest of tumor cell multiplication through (i) Enhancement of DNA blockage, (ii) Cell cycle retardation, and (iii) Mitotic activity diminution. Among these genes, the downregulation of the TOP2A gene was found to be pivotal for melanoma regression, and that this gene is highly upregulated in melanoma tissues in clinical patients with the aggressive tumour. Our investigation elucidated two classes of drugs (podophyllin derivative and anthracycline derivative) that blocks the TOP2A receptors, and could be possible therapeutic agents in melanoma patients, these drugs may have considerable potential to duplicate the process of permanent tumor regression in the clinical context.

The last phase of the thesis furnishes more rigorous insight into spontaneous cancer regression process, by contrasting melanoma tumor progression phase with melanoma tumor regression phase. Thereby, we focus on targeting two main signaling pathways of melanoma progression i.e. MAPK and PI3K/AKT signaling pathways, which we approach by inhibiting two melanoma actuating genes (BRAF and NRAS). For this methodology, microarray date of progression phase of these tumours process was analyzed. To further elucidate the mechanism, molecular docking was performed to confirm that inhibition of BRAF-NRAS oncogenes can initiate spontaneous tumour regression. We identified

Alpelisib and Cetuximab have been selected as potential repurposed drugs which conjointly inhibit both the oncogenes (and their pathways) efficiently, as we found from the aforesaid structural biology analysis. This conjoint activity may enable therapeutically replicating the process of spontaneous regression phenomenon on malignant melanoma patients.

To summarize, using mathematical modelling and systems biology methodology, we have endeavored to analyze the mechanisms behind spontaneous cancer regression which also eliminates the cancer stem cells and how normal tissue is protected during this tumor cell elimination. This study also elucidates the treatment implications, so that the mechanistic knowledge could be utilized for therapeutically replicating the tumor regression process and normal tissue protection on clinical patients. Our approach was corroborated by experimental findings from rodent, pig and human studies. The salient feature of our formulation is that the regression is enabled by temporally very specific but universal characteristics of the antitumor entities, namely single peak level of DNA impairing factor, double pulse level of white blood cell activation (T-lymphocyte), and uniform activation level of the immunomodulator cytokine (IL-2). Our formulation does not have high-intensity levels of any of the therapeutic agents for a prolonged time, their levels can become much less at intervening times, and there is no appreciable drug-induced toxicity as the normal tissue protecting immune system (circulating lymphocytes and natural killer cells) are always kept protected within physiological bounds.

Paraphrasing, the most important genes, as by enrichment analysis, are those whose downregulation prevents the growth of tumour cells by increasing DNA blockage, slowing down the cell cycle, and decreasing mitotic activity. Among these genes, TOP2A gene downregulation was discovered to be crucial for melanoma regression, and this gene is significantly overexpressed in the tissues of clinical patients with melanoma. We have identified two pharmacological classes that inhibit TOP2A receptors and may be used as

therapeutic agents in melanoma patients. These medications, called podophyllin derivatives and anthracycline derivatives, have the ability to mimic the process of tumour regression in a clinical application. It should be also noted that according to our most recent research, Alpelisib and Cetuximab have the potential to be repurposed drug that target both the melanoma signaling pathways (MAPK and PI3K/AKT) simultaneously rather than one pathway at a time.

In conclusion, the natural phenomenon of episodic permanent tumor regression is a unique biological reversal process of malignant progression, which furnishes unique signaling-pathway insight, with candidate biomolecules, which has potentiality to therapeutically replicate the tumour regression process on patients.