# Chapter 6

# Conclusions and future scope of work

# CHAPTER 6: CONCLUSION

## 6.1 Conclusion

The main objective of this thesis is to provide some insights and mechanistic basis into the naturally occurring phenomenon of spontaneous regression of cancer and to formulate a robust quantitative systems analysis biology model for it and to also find the signaling pathways related to this. Our analysis furnishes the approach of how to therapeutically induce such complete permanent tumour regression process on the patient clinically as well as delineating the molecular targets and consolidate drug molecules that could induces such a regression. We have also shown that our approach is validated by experimental findings. This chapter presents the major observations and findings of the thesis as well as its future scope as described in the following section.

**Chapter-1** introduces the basics of spontaneous cancer regression and its possible causes. A detailed literature review of spontaneous cancer regression is included, along with explanation of the drawbacks of conventional therapy and how the scientist, by understanding the mechanism of spontaneous cancer regression, help the remediation of those drawbacks. This chapter also explains the melanoma microarray based spontaneous regression investigation for the validation of our theoretical systems biology model, besides elucidating the melanoma signaling pathways. Based on the observations from literature survey, the problem statement is identified and the scope of the present study is defined.

**Chapter-2** formulates a mathematical mechanistic framework and analysis of the permanent spontaneous regression phenomenon of the malignant tumour. This extinction

of tumour cells is possible due to a negative biasing process. The major observations and finding from the chapter are summarized below:

- The regression is enabled by very specific but universal characteristics of the antitumour entities, namely (i) single peak level of DNA damaging factor, (ii) double pulse level of white blood cell activation (T-lymphocyte), and (iii) uniform activation level of the immunomodulator cytokine (IL-2).
- The second pulse feature of lymphocyte activation is an unexpected finding and accounts for the complete extinction of all the residual tumour cells which is in the order of 1% of the initial tumour load.
- The absence of this second pulse of lymphocytes in customary multimodal therapies may be a factor that prevents these therapies to induce lasting tumour eradication, and one often observes tumour relapse in these cases.
- The proposed 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 immune system (circulating lymphocytes and natural killer cells) are kept always protected, with normal physiological bounds.
- Thus, it can be taken that combinational multi-pulsed multimodal therapy, using systems biology based analysis, may offer a principled approach to permanent tumour elimination.

**Chapter-3** deals with obtaining experimental validation of our theoretical mathematical formulation of the process of permanent regression of tumour, along with delineating the clinically-relevant implications. The major observations and findings from the chapter are summarized below:

- Spontaneous melanoma regression microarray findings of pigs were investigated by Ingenuity Pathway Analysis (IPA) and we assessed the antitumour T-cell activation intensity by the level of the IPA's T-cell pathway, named "PD-1/PD-L1 cancer immunotherapy pathway". Similarly, the DNA impairment level was evaluated by the IPA pathway "G2/M DNA Damage Checkpoint Regulation". Likewise, the Natural-Killer cell level was estimated by IPA pathways "NK cell signaling". Furthermore, the Circulating lymphocyte level was assayed by the IPA pathway ("Leucocyte extravasation signaling").
- To show that our theoretically computed model of tumour regression adequately describes the experimentally observed tumour regression behaviour, the corresponding goodness-of-fit Kolmogorov-Smirnov's statistical test was satisfied ($\alpha = 5\%$).
- We also found the genes associated with the different aforesaid entities that induce the tumour regression process, namely

    - DNA Damage-related genes: CDC2, CHEK;
    - Interleukin-2 signaling-related genes: IL2RG, AKT3;
    - Natural-killer cell signaling-related genes: NKG2D, KLRK;
    - Cytotoxic T-cell activation-related genes: TRGV5, CD28;
    - Circulating lymphocyte activation-related genes: TCA, CCL5.
    - Negative bias related genes: CASP7, GZMB.
    - Cancer stem cells are also fully eliminated.

**Chapter-4** undertakes microarray-analysis to reveal the genes and signalling mechanisms involved, identifying the candidate molecules for therapeutic utility. The major observations and finding from the chapter are summarized below:

- The malignant cell extinction process occurs at the last stage of tumour regression. Here, a small negative biasing (about 1%-2% of original tumour load) eliminates the small number of residual tumour cells under the asymptotic tail of the exponentially declining curve of tumour cell population, thereby the tumour becomes permanently eradicated.
- Enrichment Analysis shows that the most significant genes are those whose downregulation produces arrest of tumour cell multiplication which occurs through (i) enhancement of DNA blockage, (ii) cell cycle retardation, and (iii) mitotic activity diminution.
- Among these genes, downregulation of TOP2A gene was found to be pivotal for melanoma regression, and this gene is highly upregulated in melanoma tissues in clinical patients.
- Two classes of drugs (podophyllin derivative and anthracycline derivative) that blocks the TOP2A receptors, could be possible therapeutic agents in melanoma patients, these drugs have a considerable potential to duplicate the process of tumour regression in the clinical context.

**Chapter-5** delineates that for obtaining more rigorous insight into spontaneous cancer regression process. We contrast melanoma tumour progression with melanoma tumour regression, and their corresponding signal transduction and gene basis. Thereby we focussed on targeting two main signalling pathways of melanoma progression, i.e. MAPK and PI3K/AKT pathways. The major observations and finding from the chapter are summarized below:

- The activation of Ras-Raf-MEK-ERK and PI3K-PKT signaling pathways occurs through either NRAS or BRAF mutations, both of which arise early during melanoma pathogenesis and are preserved throughout tumour progression.
- The candidate molecules Alpelisib and Cetuximab can be potential repurposed drugs which can target both the pathways together, instead of targeting one gene at a time, and this conjoint activity may enable therapeutically replicating the process of spontaneous tumour regression phenomenon on malignant melanoma patients.

## 6.2 Future scope of work

The future implications of the current research work are as follows:

- ❖ Our approach offers an opportunity to use combinational multi-pulsed multimodal therapy, using systems biology based analysis, and this procedure can offer a principled approach to permanent tumour elimination, without damaging the normal tissue.
- ❖ Our methodology offers up a novel prospect for incorporation of feedback controller approach to chemotherapy and immunotherapy, whereby the antitumour entities (DNA impairing drugs, interleukin-2, and lymphocytes) are accurately varied with time, such that the tumour cell population undergoes extinction in the specific time duration, by following an optimal exponentially decreasing trajectory (with negative bias).
- ❖ Our analysis also elucidates a bimodal approach to melanoma. Chapter 4 indicates the utility of general DNA blockage-inducing drugs (as Teniposide), while Chapter 5 delineates focussed melanoma-specific NRAS/BRAF-pathway interfering drugs

(as Alpelisib). Clinical trials could be undertaken to explore the possibility of enhancement of efficacy if both drugs are used together.

- The current research has clarified the modus operandi for clinical applications, by which the scientist can design infusion pump using cytologically-based tumour burden monitoring procedure (e.g., liquid biopsy in blood, or Spect imaging) at regular or weekly time intervals.