CHAPTER 5: CONCLUSIONS, NOVEL FINDINGS AND LIMITATIONS

Colorectal cancer (CRC) is one major causes of cancer-related morbidity and mortality across the globe and accounts for almost 9% of the total cancer cases identified making it a potential medical concern. The disease is the third most common cancer in men and second most common cancer in women globally. Though the disease was earlier restricted to the developed countries, in the past few years there has been a marginal increase in the incidence of CRC in developing countries mainly attributable to the change in lifestyle and dietary habits. The countries that remain at the major risk including India are the ones that are on the rise of socio-economic status and have limited health care awareness and screening programs. In India though in last few decades the incidence of CRC was lower compared to developed countries with the Age Standardized Rates (ASR) being only 4.3 and 3.5 per 100,000 males and females respectively, recent years have seen an increase in this trend which is getting closer to the incidence in developed countries. Many studies including the one by Sushmita Pathy et al. in 2012 have pointed out that the major reason for this increase in incidence is reduced intake of fruits and vegetables and increased consumption of meat and alcoholic beverages. Interestingly, the mortality rates due to CRC are lesser in developed nations despite more cases being identified mainly because of the availability of better health care system and screening programs. The major risk factors for developing CRC have been categorized into modifiable and non-modifiable factors, while the former include alcohol intake, obesity, smoking, and consumption of processed and red meat, the latter are attributed to inherited genetic alterations. Progression to CRC can be prevented in such cases by regular screening and follow-up programs. While modifiable factors are easy to address as they are more related to the life style attributes, non-modifiable factors are of major concern.

While many cancers are preventable, this is not particularly true for CRC as the disease is mostly detected in the advanced stage and remains under diagnosed until the symptoms become apparent. The survival rates for late stage CRC patients is relatively low with the rates varying between 30-60% for Stage III patients and only 3-5% for stage IV patients. Although, there are different treatment options available for the disease including surgery, chemotherapy, targeted therapy, immunotherapy etc. the success rate is limited when these regimens are used alone and are mostly associated with the secondary complications. The current treatment guidelines recommend the use of chemotherapy for early stage CRC and
targeted therapy mostly for stage IV patients. One of the major drawbacks of using chemotherapy is that it not only eradicates cancer cells but also upsets the normal cells\textsuperscript{279}. Monoclonal antibodies that are targeted only towards cancer cells offer better promise. However, there are potential limitations with the selection of targeted therapy as not all patients benefit equally and presence of predictive markers that suggest the benefit from a particular therapy are limited\textsuperscript{280}. While very few of the biomarkers are well established, there are many emerging biomarkers that can be utilized for personalization and prognosis of CRC. Though these emerging biomarkers appear promising in the small-scale studies, large clinical trials are warranted before their routine clinical application. Amongst the better studied biomarkers in the context of CRC that are predictive of benefit to anti-EGFR therapy are \textit{KRAS}, \textit{BRAF}, \textit{PIK3CA} and \textit{PTEN}. Only \textit{KRAS} from these has a well-established evidence with response to the prediction whereby patients that harbour mutations in exon 2, codon 12 or 13 do not respond to this treatment. The role of \textit{BRAF}, \textit{PIK3CA} and \textit{PTEN} as markers in the treatment of CRC still remains elusive. Even within \textit{KRAS}, though the presence of mutation indicates the lack of response, the absence of mutation does not necessarily mean a treatment success with anti-EGFR inhibitors. Thus, given the very limited utility of biomarkers in conferring a positive predictive value to available treatment regimens, it is imperative to understand the tumor biology in a more defined way and develop approaches that are more personalized and do not rely solely on the biomarker guided treatment strategies\textsuperscript{281}.

The role of EGFR in the development and progression of CRC is well established and there are several potential strategies to target it, with monoclonal antibodies and tyrosine kinase inhibitors (TKIs) being the most advanced in the clinical development\textsuperscript{282}. Of all the known mAbs, cetuximab is farthest ahead in clinical development but the key challenge that remains till date is to clearly identify which patients will show greatest response to this therapy (lack of predictive biomarkers) and the evidence of enhanced side effects and toxicity associated with higher doses of cetuximab. Efforts have been made to link EGFR over expression and gene amplification to cetuximab efficacy in CRC but has witnessed no success\textsuperscript{283}. Though the value of \textit{KRAS} and few other predictive biomarkers such as \textit{BRAF}, \textit{PIK3CA} and loss of \textit{PTEN} is established, these biomarkers are more predictive of lack of response rather than the presence of response\textsuperscript{284, 285}. Considering the potential problems associated with cetuximab administration, it is imperative to identify alternative mechanisms that can be exploited to treat CRC which is only possible by understanding the various factors that lead to the development and progression of tumor.
One of the key factors that has been noted in the progression of tumor is the accumulation of genetic alterations over time. Attempts have been made to identify these specific alterations in CRC patients, but the success has been limited mainly because each of these alterations has not been convincingly connected to the treatment modality. The deeper understanding of the genetic alterations is the mainstay for drug development and efficacy evaluation. Moreover, it is noted across different cancer types including CRC that the genetic alterations can compensate for each other to tumor’s benefit. There are compensatory mechanisms by which genetic alterations and thus signalling pathways compensate for each other which makes it critical to understand the cross-talk and dynamics between various signalling networks in the context of tumor development. One such pathway is RAS pathway which is responsible for aberrant cellular proliferation during the cancer development that has plethora of feedback loop mechanisms and is also a primary target employed by different treatment strategies in CRC. Besides genetic alterations, another limitation to combat cancer is the clonal evolution of cancer. Cancer propagates itself by clonal expansion, genetic diversification and thus clonal selection. The process of clonal selection is complicated as it involves number of mutations including driver, passenger and mutator lesions and given the premises that there are several hundreds of mutations identified it is difficult to attribute a functional relevance to each of these mutations.

Another important factor that has both beneficial and adverse effects on the development and progression of tumor is its surrounding environment referred to as tumor microenvironment (TME). It is now well understood that tumor is not a homogeneous group of malignant cells that can be studied and targeted in isolation, rather it is a complex milieu encompassing several factors such as extracellular matrix, fibroblasts, immune cells, inflammatory cells, blood vessels and lymph vessels. The interaction between these various components is both multifaceted and multidirectional with each of the component identified having a unique role in promoting the establishment and propagation of tumor. Contextually delineating the various components of TME can help design effective rationale therapeutic strategies. To maximize the effects of therapy, it is imperative to identify rationale drug combinations in a personalized setting that would maximally benefit the patient with minimal toxic effects occurring due to repeated failed treatment regimens. This study aims at delineating TME to identify various components within the tumor milieu followed by elucidating the cross-talk between these identified components to design rationale therapeutics and test them in a personalized setting termed CANscript. Understanding the key signalling pathways that either get altered or
corrupted as a process of development of CRC and other major components such as immune and metabolic compartments within the TME can provide novel rationale targeted strategies in a personalized setting. Considering the fact that tumor heterogeneity and TME can limit the predictive power of biomarker guided strategies, earlier study from our laboratory has engineered a personalized ex-vivo platform that can preserve the tumor heterogeneity and phenocopy TME, thereby enabling the testing of cancer drugs to mimic the environment within the patient’s body. The technology allows evaluating multiple drug regimens on the tumor specimen and predicting the one that would work best for the patient thereby making the entire approach tailored and personalized. The key highlight of the platform is to culture tumor in an environment that best mimics it’s native milieu by maintaining all the critical factors required for the growth of tumor. The platform considers that tumor is a complex entity rather than a simplified model of homogenous population of malignant cells as considered by many existing models including cell lines and 3D models that isolate and propagate only the tumor cells thereby neglecting the much acknowledged TME, which is critical for the tumor for its survival. Utilizing tumor stage specific tumor matrix protein (TMP) and also other autologous factors from patients we have been able to show the preservation of key signalling networks during our culture enabling us to delineate the TME while managing the signalling networks intact. It is important that oncogenic signalling cascades are maintained in totality while interrogating the drug efficacy, as it is well known that cross-talk between various signalling networks can compensate for each other and allow the tumor to propagate even in the presence of treatment modality. Thus, when the oncogenic cascade is maintained intact and efficacy of a drug is evaluated, it ensures that the cross-talk and thus the compensatory phenomenon is taken care of and does not result in ambiguity of the results obtained. Our study also demonstrated a significant ex vivo and in vivo correlation suggesting the utility of CANscript as a surrogate of animal modelling. Certain in vivo models such as PDX have been proven effective in evaluating drug efficacy, however the approach requires sub culturing and serial xenografting that takes too long, making it a difficult procedure to be followed. Further, the problem with this model is primarily the lack of immune compartment and mismatch between the derived tumor and corresponding stroma. Our model termed as CANscript has not only shown efficacy equivalent to PDX model across diverse cancer types but has also been successful in ensuring the presence of immune compartment, matched tumor and stroma with the key advantage being the short turnaround time of 7 days. The evaluation of drug regimen using the technology prevents the adverse effects in patients occurring due to administration of failed drug regimens. This platform allows testing of several
drug combinations on the tumor specimen from the patient in parallel and helps in identification of the right therapy for that particular patient making it a unique and “personalized” approach.

Our study has identified key proteins that are expressed in early and late stage CRC patients and displayed that coating the surface of culture plates with matched TMP cocktail preserves the tumor in its best possible form for up to 3 days of ex vivo culture by maintaining all the key signalling networks intact. We were able to show the preservation of tumor morphology (H&E), cellular proliferation (Ki67) and preservation of key signalling pathways (pERK1/2) in our platform after 3 days of culture similar to the baseline tumor (untreated and uncultured tumor received in the laboratory). We were also able to demonstrate the similar efficacy outcome in vivo and on CANscript platform, confirming the utility of our platform as a surrogate for animal modelling.

We utilized the established CANscript technology to identify alternative mechanisms in CRC tumors that are resistant to cetuximab despite their wild type KRAS status. While the presence of KRAS mutation confers resistance to cetuximab the presence of wild type status does not necessarily ensure a response profile. In fact, only 10-40% of the patients with wild-type KRAS status respond to cetuximab that necessitates the identification of alternate strategies that can be utilized in patients resistant to cetuximab. Towards, this end we recruited 40 clinically confirmed CRC samples and performed CANscript assays on them to evaluate the efficacy of cetuximab in these specimens (Table 7). Nine out of forty (9/40) samples were responder to cetuximab as evaluated by CANscript which is in line with the clinical setting where the overall response to CRC patients to cetuximab is about 30% (Figure 30). The study design included understanding the possible mechanism of non-response in majority of the tumors and exploring alternative rationally guided treatment strategies in these tumors. We profiled these tumors for mutations in key genes (KRAS codons 12/13, BRAF V600E and PIK3CA exon 9 and exon 20) indicated for response to cetuximab in CRC and found that out of 31 non-responder samples 4 had mutations for KRAS at codon 12 explaining their insensitivity to cetuximab treatment (Figure 32). All 9 tumors that were responsive to cetuximab were wild-type for their KRAS status. We next aimed at understanding the mechanism of resistance for remaining 27 samples.

To understand the biology of non-responsiveness in these tumors, we delineated the TME by focussing on other attributes that have been reported earlier and might be responsible for non-responsiveness to cetuximab therapy in mCRC. AREG and EREG are ligands for Epidermal
Growth Factor Receptor (EGFR) and their binding triggers the downstream signalling. We identified AREG and EREG as ligands based on published literature that might influence the response to cetuximab therapy in CRC. Low levels of AREG and EREG influence the therapy to EGFR inhibitors and make them non-responsive\textsuperscript{289}. We identified 6 out of 27 samples had low levels of \textit{AREG} and \textit{EREG} as identified by real time PCR thus explaining the potential mechanism of their non-response (Figure 34). Next, we performed a whole gene expression microarray on Agilent platform (8*60k) on 8 CRC samples that were clinically known to be responders and non-responders to cetuximab (4 responders and 4 non-responders). Results from our microarray analysis suggested that samples that were responders and non-responders to cetuximab respectively formed distinct clusters, indicating that the overall gene expression profile of responders and non-responders is significantly different from each other (Figure 35). We further performed Gene Set Enrichment Analysis (GSEA) to identify pathways that are differentially deregulated in cetuximab non-responders compared to responders (Figure 36). Although this analysis prompted out several networks including tumor cell proliferation, metabolic state, growth factor receptor and cell survival pathway, whether any of them can serve as a predictive biomarker needs further validation. Interestingly, we observed that Notch was one of the pathways with significant normalized enrichment score (NES) in non-responders compared to responders to cetuximab suggesting the possible deregulation of Notch pathway. The importance of Notch signalling in CRC has been pointed out earlier by other groups particularly due to induction of pro-survival signalling in colonic epithelial cells\textsuperscript{290}. Also, since the cross talk between EGFR and Notch is well established in several cancers including CRC, we tested the combination of cetuximab and Notch inhibitor (MK0752, an inhibitor of γ-secretase required for Notch pathway activation) in our \textit{ex vivo} setting. Another important node that was deregulated in non-responders as suggested by GSEA was Erbb2. TCGA in 2012 identified that almost 7% of CRC cases over express Erbb2 (Her2) amplification suggesting it as a potential target in CRC. Additional studies have shown that \textit{ERBB2} amplification confers resistance to cetuximab in CRC cell lines\textsuperscript{291}. This can be probably due to the compensatory mechanism of one pathway over another as both belong to the same family of receptors and considering this, we tested the combination of cetuximab and trastuzumab (a fully humanized monoclonal antibody against Erbb2) in our \textit{ex vivo} setting. None of the 21 samples responded to either MK0752 alone or the combination of MK0752 and cetuximab suggesting the presence of feedback loops and redundant pathways that promote tumor progression and survival. A very small portion of the samples responded to the
combination of trastuzumab and cetuximab (5/21), which is in line with the clinical findings by various studies and none of the samples responded to trastuzumab alone. Since, Notch and Erbb2 were identified as the pathways mainly deregulated in our non-responder cohort as identified by GSEA, we also tested a combination of MK0752 and trastuzumab without cetuximab to see if these pathways were self-sufficient to elicit a response profile. Interestingly 16 out of 21 tumors displayed a response to this combination establishing it as a novel combination in mCRC patients resistant to cetuximab (Figure 39). Our phosphoproteomic profiles combined with a high level of Notch downstream protein (HES1) highlighted the coordinated interaction of Erbb2, HES1 and Abl (Figure 37). Abl is linked to the Notch induced invasive-metastatic phenotype in CRC via reciprocal activation of DAB1, a protein induced by the Notch signaling pathway. Russo et al. and others have shown the possibility of acquisition of novel mutations for certain tumors such as MEK1<sup>K57T</sup> and KRAS<sup>Q61H</sup> which could possibly explain the resistance to the identified combination for remaining 5 tumors<sup>292</sup>. Collectively, results from this phase of the study demonstrated that there are compensatory and redundant signalling networks occurring in parallel that allow the growth and progression of tumor and molecular characterization of TME along with the utilization of a functional ex vivo platform can help identify these perturbed signalling networks within the complex milieu. The pathways so identified can be then tested in a personalized setting to evaluate the efficacy outcome.

Apart from the key signalling networks discussed above, tumor promoting inflammation along with the immunological and metabolic profile of tumors are now recognized as important players in tumor progression within the complex TME<sup>293-295</sup>. We next aimed at delineating these components of the TME and designing rationale therapeutics for patients that are otherwise non-responsive to the available treatment regimens. Towards, this end we recruited 35 samples from different clinical stages of CRC to evaluate their baseline (untreated and uncultured) immunological and metabolic profile to see if we could decipher any pattern across samples from different clinical stages (Table 8). We mainly focussed at key immune cells indicated in the progression of CRC and have a predictive value which included cytotoxic T cells (CD8), tumor associated macrophages (CD68) and regulatory phenotype (FOXP3). Since CRC is also an inflammation related disease, we also looked at the expression of key inflammatory mediators IL6 and IL8 across the samples of different clinical stages. When it came to metabolic profiling, we restricted this phase of the study primarily to glucose metabolism which is well known to be perturbed in the development of cancer and is now established as
one of the emerging hallmarks of cancer. We looked at the expression of glucose transporters (GLUT1 and GLUT4) and pAMPK expression across samples of different clinical stages to decipher the metabolic pattern (if any).

Studies by Neils Halama et al. and several other groups show that high densities of tumor infiltrating lymphocytes (TILs) suggest better prognosis independent of other prognostic factors. We evaluated the microarray data for 6 CRC samples from different clinical stages to evaluate the expression profile of already established immune signatures that included inflammasome, TAM signature, cytokine and chemokine signature. We observed a heterogeneous pattern of each of these signatures across the sample cohort suggesting the heterogeneous nature of CRC tumors from different clinical stages at a broader level. We then decided to focus independently on key immune markers. Since infiltration of CD3+ and CD8+ cells is associated with improved clinical prognosis in many cancer types including CRC and intratumoral localization of these cells also have a prognostic value, we decided to evaluate the expression profile of these cells at different clinical stages as well as between the tumor core (TC) and invasive margin (IM). Similar to large retrospective study by Gabriela Bindea et al. where the authors profiled the immunome for 105 CRC patients from different clinical stages, we observed more infiltration of CD3+ and CD8+ cells (higher median value) at the IM compared to TC. However, since our cohort was small, we did not find a statistical significance between the two. Similar to the results from the same authors we also found that the expression profile of CD3+ and CD8+ decreased with the progression of tumor from early stage to late stage. However, similar to the expression profile between IM and TC, we could not find statistical significance here which can again be attributed to the small cohort size in our study. The role of macrophages in CRC is complex as they can both promote and prevent tumor progression. Although studies such as one by Maya Gulubova et al. 2013 and Viktor Koelzer et al. 2016 suggests the presence of more CD68+ cells in the early stages of CRC tumor associated with better survival outcomes, our results indicated a heterogeneous pattern of expression of these cells with no remarkable difference between the early and late stage which could again be attributed to a small population size. Similar to CD3+ and CD8+ expression, FOXP3+ cells also had a decreased expression in later stages of CRC (albeit statistically not significant) that depict the regulatory phenotype with the immune population present in TME. This observation is in line with other studies that have been carried out to evaluate the utility of FOXP3+ cells in CRC as a prognostic factor. Thus, the immune profile between different
clinical stages of CRC tumors exhibited a heterogeneous pattern with overall relatively higher expression of CD3+, CD8+ and FOXP3+ cells in early stage tumors compared to late stage tumors. We next sought to evaluate the role of pro-inflammatory cytokines (IL-6 and IL-8) across the tumors of different clinical stages\(^{303, 304}\). Both IL-6 and IL-8 are indicated to be associated with higher expression in late stages of the diseases and thus suggesting worst prognosis. Similar to the immune expression profile observed in our study, we observed a heterogeneous pattern of expression of these inflammatory mediators across tumors of different clinical stages (Figure 46). Conclusively, delineation of immune component of TME in CRC tumors from different clinical stages displayed a heterogeneous pattern suggesting the need of evaluation of individual immune profile rather than generalizing the expression profile based on clinical stage. Our results did indicate instances where the samples had poor immune infiltration despite being in the earlier stages of the disease and vice-versa suggesting that caution should be taken, and the immune profile should be deciphered at an individual level before deciding on the course of therapy.

Cancer cells uptake glucose at a higher rate and provide lactic acid rather than metabolizing pyruvate through TCA cycle. This adaptive shift in metabolism is termed as Warburg effect and is well documented in various cancer types including CRC\(^{305}\). Overexpression of glucose transporters such as GLUT leads to acquisition of this new metabolic profile. GLUT1 is a natural transporter of glucose and is over expressed to maintain the high glycolytic rate in CRC tumors. Overexpression of GLUT1 is documented in literature associated with poor prognosis and lymph node metastasis in CRC tumors\(^{252, 306}\). Similar to GLUT1, overexpression of GLUT4 is also known to be overexpressed in CRC patients especially the ones with type-2 diabetes\(^{307}\). Considering these observations, we evaluated the expression profile of both GLUT1 and GLUT4 across CRC tumors of different clinical stages where our results indicated that both these transporters were over expressed in late stage CRC tumors compared to early stage tumors supporting the evidence for enhanced glucose requirement by cancer cells at later stage of the disease characterized by high metabolic activity needed for survival and proliferation (Figure 47). Besides these glucose transporters, AMPK has a central role in the regulation of energy metabolism in all eukaryotes\(^{308}\). The prognostic role of AMPK evaluated in several malignancies including CRC, lung, ovarian, gastric and renal cell carcinoma has suggested that except for gastric cancer, low levels of AMPK are associated with poor prognosis\(^{309}\). Our results also demonstrated that pAMPK was expressed at low levels in late stage CRC tumors compared to early stage tumors. Collectively, data from the metabolic profiling of our cohort
suggested an altered glucose metabolism in late stage CRC tumors distinct from early stage tumors as characterized by over expression of GLUT1 and GLUT4 and under expression of pAMPK in late stage tumors. We utilized this observation to design a rationale therapeutic intervention for late stage CRC tumors where we combined metformin with FOLFIRI and tested it aside each of them as single agents. Metabolism of metformin results in the activation of AMPK that can prevent the proliferation of cells validating the use of metformin as a drug of choice in CRC tumors where AMPK pathway is deregulated. Also, metformin is under evaluation in various clinical trials in combination with irinotecan, 5FU or chemoradiotherapy\textsuperscript{310, 311}.

To test our hypothesis, we recruited 10 CRC tumors from different clinical stages (4 early stage and 6 late stage). We tested metformin, FOLFIRI and the combination of two for all these samples using our established technology termed CANscript as described earlier. Our results indicated that the combination of metformin and FOLFIRI did not offer any additional benefit to CRC tumors of early stage, however, from the late stage tumors 4 out of 6 tumors responded to the combination that were otherwise non-responsive to any of the single agents (Figure 50). Currently, this study is restricted to a very small cohort and needs to be performed on a larger sample size to draw meaningful conclusions and also explore the possible mechanism by which metformin might be assisting FOLFIRI to show a pronounced effect.
NOVEL FINDINGS AND LIMITATIONS OF THIS WORK

5.1 Summary of novel findings from this work

This work aims at achieving the delineation of tumor microenvironment (TME), the importance of which has now been well understood and appreciated in tailoring our fight against cancer. The study shows that how different components within the TME interact with each other leading to a complex milieu. Within this complex microenvironment this work has primarily focussed on key oncogenic signalling networks primarily, Epidermal Growth Factor Receptor (EGFR) and related family members that are deregulated in colorectal cancer (CRC) and how understanding this cross-talk can help design rationale therapeutics. In addition to this, the study is one of its kind in establishing and utilizing an ex vivo functional platform (CANscript) that can be used to interrogate the efficacy of potential drugs that can be of benefit to the patient. Besides the oncogenic signalling network, we have also studied the immunological and metabolic parameters within the TME with special emphasis on their expression profile between the clinical early and late stage of the disease.

The findings from this study emphasize the need of personalization while evaluating the drug efficacy for patients especially in cases where positive predictive biomarkers have not been known or have limited utility. Our data also shows that tumors from different clinical stages of the disease behave differently and thus need to be addressed differently both ex vivo as well as in vivo. Data from this work shed light on the cross-talk and the compensatory mechanisms when cetuximab is used to target EGFR pathway allowing the cancer cells to sustain and proliferate. Sequential delineation of the signalling cascade prompted that Erbb2 and Notch can be co-targeted in CRC patients that are non-responsive to cetuximab despite their wild-type status for KRAS (a gene that has been approved for its negative predictive value, where by the mutated samples do not respond to cetuximab). Immunological evaluation of TME did not reveal a discrete pattern between the early and late stage tumors which warrants the study on a larger cohort. However, metabolic evaluation of TME with the special influence on glucose metabolism suggested that late stage tumors are metabolically more aggressive and using metformin in combination with that standard of care (FOLFIRI) can benefit late stage patients.
5.2 Limitations of this study and future directions

This study has its merits in being able to provide a functional platform for evaluating drug efficacy thereby saving the cost and trauma that patients typically undergo in case of a failed treatment regimen. Additionally, utilizing the functional platform, novel pathways have been identified that can be targeted in colorectal cancer. However, the findings from this study, especially the proposal of co-targeting Notch and Erbb2 needs to be evaluated at clinic as often times the combination therapy is not very well tolerated in patients. The identified combination can also be tested in a larger cohort of samples mutated for KRAS to see if this alternative strategy can also be useful for these patients, as they comprise a pretty large fraction of CRC patients (30-40%). The platform was unable to recapitulate the pharmacokinetics and toxicological behaviour of drugs or drug like molecules which play a major role in drug development and this was also not the scope of this study. We also need to have a bigger cohort to study and establish tumor-immune network and related biomarkers like CD3 and CD8 whose utility has been well established prognostically. Further, results from metabolic combination, Metformin and FOLFIRI are restricted to a very small cohort (n=10) and have not been linked to the known mutations. Exploring this combination on a larger cohort and establishing its link with the known genetic alterations can add more value to this study. Additionally, mechanism by which the combination of Metformin and FOLFIRI might potentially help the late stage CRC patients’ needs to be evaluated.

Moreover, this study evaluated all the identified combinations concurrently i.e. use of both the drugs at the same time. However, at clinic often times a drug schedule is maintained both during chemotherapy and targeted therapy where drugs are administered at specified intervals. The ex vivo platform utilized here can be modified and used to interrogate these drug combinations both concurrently and sequentially (interval between the two drugs) to see if the difference in time of introduction of drug affects the drug response pattern.