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ARTICLE TYPE

Revealing ETC-1922159 affected unknown 3^{rd} order WNT10B-X-X combinations, in silico †

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WNT10B belongs to the family of WNT proteins that are implicated in a range of phenomena that are affected by the Wnt signaling pathway. Recent studies have shown that WNT10B plays a role in colorectal cancer. WNTs have been found to directly affect the stemness of the tumor cells via regulation of ASCL2. Switching off the ASCL2 literally blocks the stemness process of the tumor cells and vice versa. Furthermore, recent findings suggest BVES to be highly suppressed in malignancies and in vitro deletions of BVES show higher Wnt signaling activity to induce stemness. WNT10B was found to be highly expressed in such cases. Often, in biology, we are faced with the problem of exploring relevant unknown biological hypotheses in the form of myriads of combination of factors that might be affecting the pathway under certain conditions. For example, WNT10B-ASCL2 is one such 2^{nd} order combination whose relation needs to be tested under the influence of recently developed porcupine-WNT inhibitor ETC-1922159. The inhibitor is known to suppress PORCN (porcupine) and thus inhibit a range of oncogenes known to be directly or indirectly affected by the Wnts. In a recent unpublished work in bioRxiv, Sinha[1], we had the opportunity to rank these unknown biological hypotheses for down regulated genes at 2^{nd} order level after the drug was administered. The in silico observations showed that the combination of WNT10B-ASCL2 was assigned a relatively lower rank, thus validating the pipeline's efficacy with the confirmed wet lab experiment that indicate that both WNT10B and ASCL2 were down regulated after treatment in cancer cells. Here, we take one step further by in silico analysis of the 3^{rd} order combinations of WNT10B-X-X (X can be known or unknown factor), from a range of 100 randomly picked down regulated genes after ETC-1922159 treatment. The pipeline uses the density based HSIC (Hilbert Schmidt Information Criterion) sensitivity index with an rbf (radial basis function) kernel, which is known to be highly effective in sensitivity analysis. Various unknown/unexplored/untested 3^{rd} order biological hypotheses emerge some of which are confirmed in wet lab, while others need to be tested. The potential of such ranking is indispensable in the current era of search in a vast combinatorial forest. **KEYWORDS** - WNT pathway; porcupine inhibitor ETC-1922159; sensitivity analysis; colorectal cancer; unknown biological hypotheses; combinatorial search space; support vector ranking

WORKING DRAFT IN PROGRESS

Significance

WNT10B belongs to the family of WNT proteins that are implicated in a range of phenomena that are affected by the Wnt signaling pathway. Recent studies have shown that WNT10B plays a role in colorectal cancer. Often, we are faced with the problem of exploring relevant unknown biological hypotheses in the form of myriads of combination of factors that might be involved in the pathway. The current work reveals at in silico level, the 3rd order

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Worked as independent researcher

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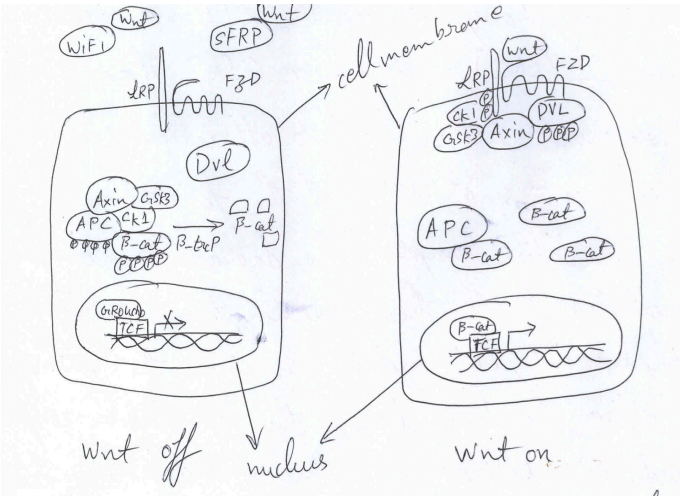

Fig. 1 Cartoon of Wnt Signaling from [1].

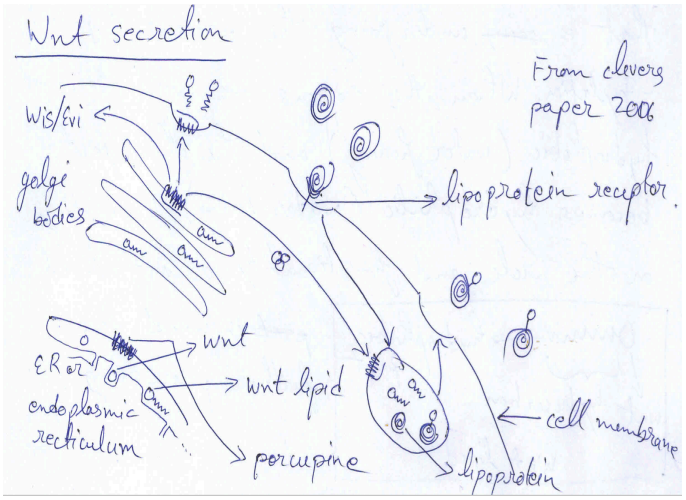

Fig. 2 Cartoon of Wnt Secretion from [1].

WNT10B associated combinations that might be affected by the administration of Porcupine-Wnt inhibitor drug ETC-1922159 in colorectal cancer cells. The potential of revealing such higher order combinations via ranking is indispensable in the current era of search in a vast combinatorial forest.

We reproduce a part of the manuscript [1] before we delve into the details of the current work.

Introduction

Wnt signaling and secretion

[2]'s accidental discovery of the Wingless played a pioneering role in the emergence of a widely expanding research field of the Wnt signaling pathway. A majority of the work has focused on issues related to • the discovery of genetic and epigenetic factors affecting the pathway [3] &[4], • implications of mutations in the pathway and its dominant role on cancer and other diseases [5], • investigation into the pathway's contribution towards embryo development [6], homeostasis [7] &[8] and apoptosis [9] and • safety and feasibility of drug design for the Wnt pathway [10], [11], [12], [13] &[14].

The Wnt phenomena can be roughly segregated into signaling and secretion part. The Wnt signaling pathway works when the WNT ligand gets attached to the Frizzled(FZD)/LRP coreceptor complex. FZD may interact with the Dishevelled (DVL) causing phosphorylation. It is also thought that Wnts cause phosphorylation of the LRP via casein kinase 1 (CK1) and kinase GSK3. These developments further lead to attraction of Axin which causes inhibition of the formation of the degradation complex. The degradation complex constitutes of AXIN, the β-catenin transportation complex APC, CK1 and GSK3. When the pathway is active the dissolution of the degradation complex leads to stabilization in the concentration of β-catenin in the cytoplasm. As β-catenin enters into the nucleus it displaces the GROUCHO and binds with transcription cell factor TCF thus instigating transcription of Wnt target genes. GROUCHO acts as lock on TCF and prevents the transcription of target genes which may induce cancer. In cases when the Wnt ligands are not captured by the coreceptor at the cell membrane, AXIN helps in formation of the degradation complex. The degradation complex phosphorylates β-catenin which is then recognised by F BOX/WD repeat protein β-TRCP. β-TRCP is a component of ubiquitin ligase complex that helps in ubiquitination of β-catenin thus marking it for degradation via the proteasome. A cartoon of the signaling transduction snapshot is shown in figure 1.

Contrary to the signaling phenomena, the secretion phenomena is about the release and transportation of the WNT protein/ligand in and out of the cell, respectively. Briefly, the WNT proteins that are synthesized with the endoplasmic reticulum (ER), are known to be palmitoyleated via the Porcupine (PORCN) to form the WNT ligand, which is then ready for transportation [15]. It is believed that these ligands are then transported via the EVI/WNTLESS transmembrane complex out of the cell [16] &[17]. The EVI/WNTLESS themselves are known to reside in the Golgi bodies and interaction with the WNT ligands for the later's glycosylation [18] &[19]. Once outside the cell, the WNTs then interact with the cell receptors, as explained in the foregoing paragraph, to induce the Wnt signaling. Of importance is the fact that the EVI/WNTLESS also need a transporter in the from of a complex termed as Retromer. A cartoon of the signaling transduction snapshot is shown in figure 2.

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WNT10B

WNT10B has been found to be implicated in a range of cancers. In gastric cancer, the knockdown of WNT10B showed reduced expression of cell proliferation and migration as well as inhibition of epithelial-mesenchymal transition[20]. On the other hand, WNT10B is also involved in the formation of bone mass and progenitor maintenance of various kinds of tissue, while deletion of the same leads to loss of bone mass and mesenchymal progenitor cells[21]. Their contribution is also reported in axonal regeneration in injured CNS[22]. Furthermore, like WNT10B, WNT10A and WNT6 have shown to play a major role in inhibiting adipogenesis and stimulates osteoblastogenesis while regulating the mesenchymal stem cells[23] &[24]. Involvement in heptocellular carcinoma of WNT10B has been found wherein it is shown that stable silencing of WNT10B leads to significant reduction in proliferation, colony formation, migration and invasion in HepG2 HCC cell line[25]. Its implication in breast cancer[26] &[27] as well as endometrial cancer [28] has also been reported.

In colorectal cancer, WNT10B has shown to play a dual function of both oncogenesis promotion via β-catenin/TCF pathway and the inhibition of cell growth, possibly via FGF family of proteins [29]. Methylation of WNT10B has been found in the some of the cancer cell lines while its reversal has lead to over-expression of the WNT10B. However, the over-expression of WNT10B has lead to reduced cell growth in cancer, indicating a β-catenin independent component to be behind such a phenomena. Methylation of over-expressed WNT10B and synergistic work with FGF family of proteins later indicate the promotion of oncogenesis, as has been demonstrated in[29].

In a more recent work, ASCL2 has been found to play a major role in stemness in colon crypts and is implicated in colon cancer[30]. Switching off the ASCL2 leads to a literal blockage of the stemness process and vice versa. At the downstream level, ASCL2 is regulated by TCF4/β-catenin via non-coding RNA target named WiNTRLINC1[31]. Activation of ASCL2 leads to feedforward transcription of the non-coding RNA and thus a loop is formed which helps in the stemness and is highly effective in colon cancer. At the upstream level, ASCL2 is known act as a WNT/RSPONDIN switch that controls the stemness[32]. It has been shown that removal of RSPO1 lead to decrease in the Wnt signaling due to removal of the FZD receptors that led to reduced expression of ASCL2. Also, low levels of LGR5 were observed due to this phenomena. The opposite happened by increasing the RSPO1 levels. After the drug treatment, it was found that ASCL2 was highly suppressed pointing to the inhibition of stemness in the colorectal cancer cells. Also,[32] show that by genetically disrupting PORCN or inducing a PORCN inhibitor (like IWP-2), there is loss of stem cell markers like LGR5 and RNF43, which lead to disappearance of stem cells and moribund state of mice. A similar affect can be found with ETC-1922159, where there is suppression of RNF43 and LGR5 that lead to inhibition of the Wnt pathway and thus the ASCL2 regulation. These wet lab evidences are confirmed in the relatively low ranking of the combination ASCL2-RNF43 via the inhibition of PORCN-WNT that leads to blocking of the stemness that is induced by ASCL2. Since ASCL2 is directly mediated by the WNT proteins, the recorded ASCL2-WNT10B combination showed low priority ranking of 488, 497 and 321 for rbf, laplace and linear kernels, respectively, thus indicating a possible connection between WNT10B and ASCL2 activation. WNT10B might be playing a crucial role in stemness. This is further confirmed by wet lab experiments in[33], which show BVES deletion results in amplified stem cell activity and Wnt signaling after radiation. WNT10B has been implicated in colorectal cancer[29].

PORCN-WNT inhibitors

The regulation of the Wnt pathway is dependent on the production and secretion of the WNT proteins. Thus, the inhibition of a causal factor like PORCN which contributes to the WNT secretion has been proposed to be a way to interfere with the Wnt cascade, which might result in the growth of tumor. Several groups have been engaged in such studies and known PORCN-WNT inhibitors that have been made available till now are IWP-L6[34] &[35], C59 [36], LGK974[37] and ETC-1922159[38]. In this study, the focus of the attention is on the implications of the ETC-1922159, after the drug has been administered. The drug is a enantiomer with a nanomolar activity and excellent bioavailability as claimed in[38].

Combinatorial search problem and a possible solution

We have already addressed the issue of combinatorial search problem and a possible solution in[39] and[1]. The details of the methodology of this manuscript have been explained in great detail in[39] & its application in[1] and readers are requested to go through the same for gaining deeper insight into the working of the pipeline and its use for published data set generated from ETC-1922159. In order to understand the significance of the solution proposed to the problem of combinatorial search that the biologists face in revealing unknown biological search problem, these works are of importance. Using the same code with minor modifications in[39] and[1], it was possible to generate the rankings for 3^{rd} order combinations. 100 genes were randomly selected from the list of down regulated genes, by the pipeline and a 3^{rd} order combination was generated from those 100 genes. The total number of gene combination with $C_3^{100} = 161700$. Out of these the WNT10B associated 3^{rd} order combinations were selected, which account to a total of 4851 combinations. The goal of this manuscript is to analyse these 3^{rd} order ranked WNT10B associations.

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Results & discussion

We present here the 3^{rd} order combinations associated with the WNT10B and represent them as WNT10B-X-X were X can be known or unknown factor from a list of genes that were affected after the administration of the ETC-1922159 drug. There are a total of 4851 combinations of randomly selected 100 genes from a list of 2500± genes. Out of these 100, WNT10B was one of them. Here we analyse some of the ranked combinations out of 4851 3^{rd} order interactions. Note that the rankings were generated using only the HSIC density index using the radial basis function kernel. Also, the rankings for a particular gene might change over different combinations and the biologists/oncologists are advised to cross check across the different tables presented. However, where possible, we report confirmatory results by the pipeline that fall in line with the published and known mechanism of a particular gene under consideration. Also, many of the combinations are yet to be tested and we make openings for the deeper analysis and exploration of the combinations as future work.

SCO1-WNT10B-X combinations

The most important functionality of mitochondria is the production of ATP through respiration process and the regulation of the cellular metabolism. Illingworth[40] in a tutorial indicate - " The study of mitochondrial functionality is usually done via toxic compounds. Inhibitors help in distinguishing the electron transport system from the phosphorylation system and help in defining the redox carriers along the respiratory chain. If the chain is blocked then all the intermediates on the substrate side of the block become more reduced, while all those on the oxygen side become more oxidised. It is easy to see what has happened because the oxidised and reduced carriers often differ in their spectral properties. There are six kinds of poisons which might affect the mitochondrial functioning - • respiratory chain inhibitors • phosphorylation inhibitors • uncoupling agents • transport inhibitors • ionophores and • Krebs cycle inhibitors." The distribution of copper and its homeostasis plays a major role in many biological processes[41] and this is facilitated by the work of metal transporters and chaperones[42,43] &[44]. Disruption in pathways that transport copper can cause major damages in the form of metallostasis found in tumors[45]. The pathway is regulated by copper homeostasis genes and recent transcriptome analysis of copper homeostasis genes have shown the upregulation of SLC31A1, SCO and COX11 in colorectal cancer cases[46].

It has been shown that human SCO1 and SCO2 have independent cooperative functions in copper delivery to cytochrome c oxidase (CcO)[47,48] &[49]. CcO is a multimeric protein complex with 13 subunits working in tandem to transfer electrons from reduced cytochrome c to molecular oxygen in the terminal step of the respiratory chain. It is embedded in the inner mitochondrial membrane (IMM) and composed of three mitochondrially encoded subunits (COX I - COX III) and ten nuclear encoded subunits. Additional factors are required for the proper functioning of the complex. Mutations in SCO1 and SCO2 that code for metallochaperone proteins with roles of copper delivery to COX, lead to severe deficiencies in the respiratory mechanism. Leary *et al.*[47] show that overexpression of SCO1 in a SCO2 patient background, and vice versa, has a dominant-negative effect on COX activity, indicating the non-overlapping functions of SCO1 and SCO2. Finally, Leary *et al.*[47] propose a model were COX17 a metallochaperone, transfers copper to SCO2, which in turn delivers it to COX II. SCO1 facilitates the latter interaction, thereby promoting the biogenesis of the copper site. The metallation of COX II occurs at an early stage of COX assembly and is required for the incorporation of this structural subunit into the assembling holoenzyme. To confirm the matter, different configurations of SCO1 have been found to be existant while interacting with COX II, for copper transfer[50].

Armed with this information we begin with the analysis of the combinations of SCO1-WNT10B-X (X a known or unknown combination) that is presented in table 1. Since it is known that both WNT10B and SCO1 are implicated and upregulated in colorectal cancer cases, low rankings of these match with the fact that after the administration of the drug ETC-1922159 WNT10B and SCO1 were suppressed. In a majority of the rankings that we observe in table 1, most of them are assigned a very low rank (nearing to 1) in the randomly chosen list of down regulated genes. We analyse the functions of the 3^{rd} component X which might be either a known factor or an unknown factor.

X - known/unknown/untested factor with SCO1-WNT10B

Madan *et al.*[51] report that both WNT10B and SCO1 were down regulated by the administration of ETC-1922159. Out of the 100 randomly selected genes, XX172, an unknown factor was found to have a very low ranking of 1 by the pipeline. This points to the fact that this unknown factor is suppressed by the drug and can be a major factor in the propagation of colorectal cancer. The pipeline reveals its strength by pointing towards this unknown and unexplored factor and gives the oncologists/biologist an insight into the newly observed factor that might be contributing heavily in the colorectal cancer and is found to be highly suppressed after the drug treatment. Further wet-lab test might reveal major implications regarding XX172. Similarly, the unknown factors XX91 (ranked 21), XX81 (ranked 31), XX134 (ranked 51), XX16 (ranked 77), XX148 (ranked 171) and XX228 (ranked 199) out of the 4850± combinations of WNT10B-SCO1 combinations in randomly selected 100 genes show a similar behaviour and require further wet lab investigations based on the pipeline's in-

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RANKING USING HSIC - RBF KERNEL W.R.T WNT10B-SCO1

3^{nd} odr comb.	rbf rank	3^{nd} odr comb.	rbf rank	3^{nd}odrcomb.	rbf rank
SCO1-WNT10B-XX172	1	SCO1-WNT10B-GFRA3	2	SCO1-WNT10B-TXLNG	3
SCO1-WNT10B-DHX9	4	SCO1-WNT10B-ABCA2	5	SCO1-WNT10B-PABPC1L	6
SCO1-WNT10B-LEFTY1	8	SCO1-WNT10B-TTC26	9	SCO1-WNT10B-WDR76	10
SCO1-WNT10B-UHRF1	12	SCO1-WNT10B-TFB2M	15	SCO1-WNT10B-ACVR1C	18
SCO1-WNT10B-IPO9	19	SCO1-WNT10B-LPAR6	20	SCO1-WNT10B-XX91	21
SCO1-WNT10B-CDCA2	23	SCO1-WNT10B-LCTL	27	SCO1-WNT10B-DEPDC1B	28
SCO1-WNT10B-EXOSC5	30	SCO1-WNT10B-XX81	31	SCO1-WNT10B-RCN2	32
SCO1-WNT10B-COLEC11	35	SCO1-WNT10B-POLA2	36	SCO1-WNT10B-CDK5RAP2	38
SCO1-WNT10B-FZD7	39	SCO1-WNT10B-SGOL1	40	SCO1-WNT10B-TP73	41
SCO1-WNT10B-RAB40A	42	SCO1-WNT10B-TGIF2	43	SCO1-WNT10B-PDCD7	45
SCO1-WNT10B-GPX1	47	SCO1-WNT10B-ZNF572	48	SCO1-WNT10B-KIF20B	49
SCO1-WNT10B-XX134	51	SCO1-WNT10B-SLC7A8	52	SCO1-WNT10B-IL17D	55
SCO1-WNT10B-ING5	56	SCO1-WNT10B-MNS1	57	SCO1-WNT10B-DDN	61
SCO1-WNT10B-CBX5	64	SCO1-WNT10B-C4orf46	69	SCO1-WNT10B-FAM131B	70
SCO1-WNT10B-RRM1	71	SCO1-WNT10B-FOXD2.AS1	72	SCO1-WNT10B-MARVELD1	75
SCO1-XX16-WNT10B	77	SCO1-WNT10B-NUBPL	78	SCO1-WNT10B-TAMM41	80
SCO1-WNT10B-ARHGAP11B	85	SCO1-WNT10B-RRS1	89	SCO1-WNT10B-PPT1	91
SCO1-CTTNBP2-WNT10B	93	SCO1-WNT10B-MEGF8	97	SCO1-WNT10B-IPO11	103
SCO1-HADH-WNT10B	107	SCO1-WNT10B-ATAD3A	110	SCO1-WNT10B-DEPDC7	119
SCO1-TIMM9-WNT10B	124	SCO1-WNT10B-TARS2	133	SCO1-WNT10B-MTHFD2L	136
SCO1-WNT10B-HNRNPC	137	SCO1-WNT10B-ZNF502	141	SCO1-GINS3-WNT10B	149
SCO1-WNT10B-METTL16	151	SCO1-WNT10B-MTHFD1L	157	SCO1-WNT10B-SEC31B	159
SCO1-WNT10B-XX148	171	SCO1-WNT10B-METTL12	174	SCO1-WNT10B-BOLA3	188
SCO1-FAM168A-WNT10B	191	SCO1-WNT10B-EXOSC3	195	SCO1-WNT10B-XX228	199
SCO1-TUBA1B-WNT10B	202	SCO1-WNT10B-ACTL6A	216	SCO1-GCFC2-WNT10B	226
SCO1-WNT10B-IRF8	227	SCO1-WNT10B-PAXIP1.AS2	228	SCO1-WNT10B-NUP160	236
SCO1-WNT10B-CD3EAP	256	SCO1-WNT10B-ZNF594	268	SCO1-XPOT-WNT10B	276
SCO1-WNT10B-SNHG16	280	SCO1-WNT10B-TGFB1	298	SCO1-WNT10B-TBRG4	336
SCO1-WNT10B-DNASE2	354	SCO1-WNT10B-CAAP1	410	SCO1-WNT10B-ELAC2	424
SCO1-SMC1A-WNT10B	438	SCO1-WNT10B-SELENBP1	516	SCO1-WNT10B-ZNF740	541
SCO1-WNT10B-VPRBP	656	SCO1-WNT10B-CBR1	1918	SCO1-WNT10B-KARS	2793
ARL9-SCO1-WNT10B	3196	ODF2-SCO1-WNT10B	4512	PDE7A-SCO1-WNT10B	4728
CEP78-SCO1-WNT10B	4844	RETNLB-SCO1-WNT10B	4850		

Table 1 3^{rd} order interaction ranking using HSIC for radial basis function kernel. Total number of 3^{rd} order interactions in a set of 100 genes - 161700. 4851 3^{rd} order combinations for WNT10B associated work. Rankings for SCO1-WNT10B-X have been tabulated.

dication.

Mechanism of amplification of oncogenes is a property of many of the tumors. DM or double minute chromosomes are DNA segments containing amplified oncogenes and their frequency is high in tumor cases. Ji *et al.*[52] observe the amplification of ZNF572 in colorectal cancer cases via these DMs. After the administration of ETC-1922159 in different colorectal cancer cases, ZNF572 is found to be down regulated and its rankings along with SCO1-WNT10B is found to be extremely low (48) in the randomly selected set of 100 genes.

Chemotherapeutic treatment of metastatic CRC is usually based on combination of 5FU antimetabolite drug and DNA binding agent Oxaliplatin. Jensen *et al.*[53] observed that ZNF502 was found to be down regulated in Oxaliplatin resistant cell lines indicating that associated gene that is resistant the drug in cancer cell line. However, Bash-Imam *et al.*[54] observed the upregulation of ZNF502 after treatment with $10\mu M$ of 5-FU in HCT-116 cells. After the treatment of ETC-1922159, the ZNF502 was found to be downregulated. Its combination with SCO1-WNT10B was confirmed to have lower rank (141) indicating possible direct or indirect combinatorial play in the Wnt pathway during the cancer stages. Similar interpretations could be found for ZNF594 and

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ZNF740.

RET is a member of GDNF family receptor complex and is a receptor for the different kinds of ligands. It binds the ligands to form a multi-receptor complex that includes GFRα (GFRA) proteins to activate receptor tyrosine kinases in a variety of signaling. However, it has been found to be a tumor suppressor in colorectal cancer case and is methylated[55]. Moreover, the functional RET receptor complex includes RET and one of four glycosylphosphatidylinositol-anchored co-receptors, designated GDNF-α receptors, GFRα1, GFRα2, GFRα3 (GFRA3), and GFRα4 and Luo *et al.*[55] observe that GFRA3 is expressed in the colorectal cancer cases. GFRA3 was found to be downregulated on treatment with the ETC-1922159 and might have been highly expressed in the colorectal cancer case. Its consequent downregulation along with SCO1-WNT10B gets one of the lowest priority of 2 in a randomly selected set of 100 genes.

DHX9 is a RNA helicase that belongs to the family of DExD/H-box family and is found to be involved in the transcriptional regulation in cancer[56] &[57]. DHX9 has been found to be upregulated in colorectal cancer cases and after the treatment of the ETC-1922159, it was downregulated. The combination of SCO1-WNT10B-DHX9 acquires lower priority of 4, indicating a possible potential synergistic affect (within the selected 100 genes).

ABCA2 belongs to the category of ABC transporters that play an essential role in the development of resistance by the efflux of anticancer agents outside of cancer cells[58]. Hlavata *et al.*[58] observed that ABCA2 had no significant change/affect in colorectal cancer cases. Kobayashi *et al.*[59] found ABCA2 to be downregulated in colorectal cancer case. Contrary to this, ETC-1922159 affected cancer cells showed down regulation of ABCA2 and the pipeline points towards the low rank (of 5) associated with the combination of WNT10-SCO1. This entails further investigation in wet lab regarding the functionality of ABCA2.

PABPC1L (as recorded by[51]) or the cytoplasmic 1-like poly(A)-binding protein[60], belong to a family of multifunctional proteins PABPs which regulate and stabilize the mRNA translation. They are observed to be helpful in the transportation of the mRNA also from the nucleus and there exists a nucleic version of PABP also[61]. PABPC1 contains four non identical RRMs that are joined with the main PABC domain and separated by a linker. Though their mechanism of import and export of mRNA has not been understood deeply, a few models/observations have been made. Association with translation complexes or mRNA at cytoplasmic level inhibits the transportation of PABPC1 to the nucleus. Contrary to this, its release leads to bindation with Importin-α/β complex that facilitates in nuclear import. There are various mechanisms by which PABPC1L might be exported out of the nucleus. Modes of export involve association with (1) cytoplasmic eEF1α (2) mRNA as well as TAP that mediates the mRNA export and (3) Paxillin via CRM1 pathway. In gastric cancer cases, PABPC1 has been found to be an oncoprotein[62] and observed to exert carcinogenesis. In colon cancer mutations in PABPC1 have been found in minor tumor clones[63]. Genomic correlates of immune-cell infiltrates in CRC have found the existence of significantly mutated CRC genes[64]. PABPC1L was found to be downregulated after the treatment of ETC-1922159 and a low ranking (of 6) is associated with SCO1-WNT10B. In CRC, PABPC1L might be mutated and work as oncoprotein and facilitate the transmission of other oncogenic factors. The administration of the drug down regulated the gene in drug treated CRC cells and the low ranking confirms at in silico level the suppression at cytoplasmic level. Additionally, since RRM1 is linked to the PABPs, the pipeline found a similar low ranking (of 71) with the combination SCO1-WNT10B (See table 1). Discussion on RRM1 combination will be done a little later.

LEFTY1 belongs to the family of LEFTY genes involved in the left-right determination[65]. Yashiro *et al.*[65] study the LEFTY pair for human cases in colon. They show that the LEFTY1 is highly expressed in colorectal cancer as well as normal colon and point that it is not easy to correlate the two phenomena. Naba *et al.*[66] also identify LEFTY1 in primary colon tumor signatures. After the ETC-1922159 drug, LEFTY1 was found to be down regulated and the pipeline shows this confirmatory result by assigning a low rank (of 8) to LEFTY1 along with SCO1-WNT10B. Again, association of SCO1-WNT10B-LEFTY1 needs to be explored at wet lab level.

Tetratricopeptide repeat protein 26 (TTC26, also known as Intraflagellar transport protein 56), has found to be involved in cilia formation in zebrafish[67]. It has been found to impair hedgehog signaling on mutation[68]. Not much is known about TTC26 in case of colorectal cancer and after the ETC-1922159 treatment, it was found to be down regulated. The pipeline shows a very low ranking (of 9) in a set of 100 genes which confirms the wet lab results on drug treatment and further investigation of the role of TTC26 in colorectal cancer is required.

CMR1 is found to be involved in response to DNA replication stress which contributes to genomic instability. Gallina *et al.*[69] also observe that, its human orthologue, WDR76 responds to DNA damage via association with CCT to recover from genomic instability via regulation of turnover of sumoylated and phosphorylated proteins. Human WDR76 is known to interact with XRCC-5/6 which are known to mediate or stabilize RAD51 during the homologous recombination (HR) process. WDR76 was found to be down regulated by the ETC-1922159 treatment and the pipeline assigned a low ranking of 10. This suppression of the WDR76 indicates the drug's affect in destabilizing the colorectal cancer cells whose stability might be sustained by the WDR76 or its mutated version which might have lead to propagation of CRC, has been suppressed. Further investigation is necessary.

Recently, UHRF1 is a newly found gene that translates to (Ring)-finger domain containing associated protein which is re-

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quired for the survival and tumorigenicity[70]. Taniue *et al.*[70] show that UPAT a long noncoding RNA UPAT interacts with UHRF1 to interfere with the β-TRCP that is involved in the ubiquitination process. This interaction is basically the inhibition of the degradation of UHRF1. Wang *et al.*[71] also show that UHRF1 is upregulated in colorectal cancer case and facilitates in cell proliferation and metastasis via suppression of $p16^{ink4a}$. Similar findings have been made in[72],[73] &[74]. Consistent with these, the UHRF1 was down regulated in cancer cells, on the treatment with ETC-1922159. The pipeline confirms this with a low rank of 12 along with WNT10B-SCO1 combination.

Mitochondrian DNA transcription happens via the phenomena of the formation of the D-Loop structure[75]. The transcription requires mitochondrial RNA polymerase and Tfam, a DNA binding stimulatory factor. In presence of this Tfam and the mitochondrial RNA polymerase, the mitochondrial transcription specificity factors TFB1M and TFB2M enhance the transcription. Gleyzer *et al.*[76] show that these are further controlled by NRF-1/2 and PGC-1 coactivators. Furthermore, Cyclophilin-D is found to interact with TFB2M for mitochondrial transcription[77]. TFB1M and TFB2M is found to be significantly reduced on FOXO3a activation[78]. While in colorectal cancer cases FOXO3a is found to be highly inhibited[79] &[80]. In colorectal cancer cases TFB2M is found to be upregulated and after the ETC-1922159 treatment, it was found to be down regulated. The low ranking of 15 of TFB2M by the pipeline with WNT10B-SCO1 suggests the effectiveness of the framework to assign a low priority to the suppression of TFB2M by the drug.

It has been found that Nodal promotes the self-renewal of human colon cancer stem cells[81] and it signals through activation of receptor complex, including ALK-4/7. ALK-7 is the protein that is encoded by ACVR1C. ACVR1C plays a major role for embryogenesis and left-right patterning in the mouse[82]. However, Nagaoka *et al.*[83] show that the modulation of the β-catenin pathway by Cripto-1 in response to Wnt3a stimulation is independent of the Nodal/Alk4/Alk7 signaling pathway. Nevertheless, ACVR1C is known to be activated in colorectal cancer case as has been observed in[81]. After the administration of ETC-1922159, ACVR1C was found to be down regulated and the pipeline assigns this down regulation with a low priority of 18.

Importin-β family proteins are transport receptors that help in the transportation of proteins and RNAs in and out of the nucleus via the nucleus pores. Kimura and Imamoto[84] give a detailed review of the importins. It was observed that Importin-α/β was responsible in the transportation of PABPC1L (the cytoplasmic 1-like poly(A)-binding protein[60] that belong to a family of multifunctional proteins PABPs which regulate and stabilize the mRNA translation. The ranking of the PABC1L was found to be low after its suppression by ETC-1922159. Since Importin-α/β facilitate the transportation of PABPC1L, it is likely that it is also suppressed after the drug treatment. Furthermore, Importin-9 is implicated in transport of ARID3A into the nucleus where it forms a complex with ARID3B which is responsible for stemness in cancer. Liao *et al.*[85] show that deletion of LET7 which suppresses ARID3A and Importin-9 and is thus a tumor suppressor, leads to initiation of cancer via ARID3B. In their experiment[51], show that the drug treatment suppresses Importin-9 that is IPO9 and thus the stemness property of the colorectal cancer cases. This is indicated by the pipeline with a low rank of 19. Similarly, IPO11 gets a low rank of 103.

Lysophosphatidic acid receptors (LPAR6) are commonly overexpressed in HCC and supports tumorigenicity in the same[86]. LPAR are known to be implicated in tumor metastasis and p2y5 has been found to be a LPAR and was designated as LPAR6[87]. Not much is known about the LPAR6 in colorectal cancers and it was found to be down regulated after the administration of the drug. The pipeline correlates with the down regulation of the LPAR6 and shows a ranking of 20 after the drug treatment.

Uchida *et al.*[88] show on knockdown, that CDCA2 or Cell division cycle associated 2 inhibit the cellular proliferation by arresting cell-cycle progression at G1 phase and upregulating the cyclin-dependent kinase inhibitors ($p21^{cip1}$, $p27^{kip1}$, $p15^{ink4b}$, and $p16^{ink4a}$), in human squamous cell carcinoma. Earlier, we saw that also Wang *et al.*[71] show that UHRF1 is upregulated in colorectal cancer case and facilitates in cell proliferation and metastasis via suppression of $p16^{ink4a}$. It is evident that overexpression of CDCA2 leads to repression of $p16^{ink4a}$ and cell proliferation. Consistent with these, Kwon *et al.*[89] show that CDCA2 is upregulated in the colorectal cancer case. Wang *et al.*[90] also show the upregulation of CDCA2 in recurrent and non-recurrent types of CRC. After the treatment of ETC-1922159 in the subtype of colorectal cancer cells, CDCA2 was down regulated and facilitated in inhibition of growth of cancer cells[51]. This confirmatory result has been indicated by the pipeline with a rank of 23.

Matsumura *et al.*[91] identified that the inactivation of the Klotho (KI) gene in mice showed disorders that resemble human aging. Knockdown of Klothoγ (KLG) or LCLT has been implicated in immortalization of normal human colonic epithelial cells [92]. Reversibly, this means that overexpression/up-regulation of LCLT would not immortalize the colonic cells. Kim *et al.*[92] show that the KLG/LCTL knockdown lead to disappearance of KLA (Klothoα) which is a tumor suppressor and canonical Wnt antagonist. In colorectal cancer, LCLT might be highly regulated and after the ETC-1922159 administration, the LCLT was found to be downregulated. The pipeline points to the suppression of LCLT by assigning a low ranking of 27.

Liao *et al.*[93] show via machine learning methods that DEP (Dishevelled/EGL-10/Pleckstrin) domain containing (DC) proteins are expressed in HCC. It has been found to be highly expressed in colorectal cancer[94] and[95]. After the administration

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of the ETC-1922159, DEPDC1B and DEPDC7 were down regulated and the pipeline indicates a low rank of 28 and 119 for the same down regulation, respectively. Exosome is a highly conserved complex that mediates the degradation and processing of multiple classes of RNA Liu *et al.*[96]. It is composed of 9 subunits marked EXOSC-1/2/.../9. In cancer cells it might be that the exosome is disrupted from its execution of the degradation of the multiple tumor causing RNAs. EXOSC3 (RRP40) and EXOSC5 (RRP46) show low rank of 195 and 30 and were found to be suppressed after the treatment of ECT-1922159.

ZIC1 (Zinc finger of the cerebellum) is found to be highly suppressed via hypermethylation in colorectal cancer and is known to be a tumor suppressor Gan *et al.*[97]. However, in breast cancer cases as Nakakido *et al.*[98] show, the expression of ZIC1 is found to be regulated by the knockdown of PIGX as well as RCN1 (reticulocalbin 1) and (reticulocalbin 2) RCN2. Thus upregulation or overexpression of RCN2 negatively regulates ZIC1 for cancer proliferation. RCN2 was found to be overexpressed in gastrointestinal cancer cells lines[99]. After treatment with ETC-1922159 in colorectal cancer samples, RCN2 was found to be down regulated and the pipeline assigns this down regulation to a rank of 32 along with WNT10B-SCO1.

COLEC11, has been found to be differentially expressed in colorectal tumor cases[100]. Not much is known about COLEC11 in colorectal cancer and it was found to be down regulated after the treatment of ETC-1922159 drug and indicated with a low ranking of 35 along with WNT10B-SCO1 combination.

Binding of SRL (Sclerotium rolfsii lectin) to human colon has been found to induce cell apoptosis and suppression of tumor growth[101]. SRL treatment also downregulated POLA2[101]. POLA2 encodes DNA polymerase α subunit 2 and pairs with PARP to do a DNA damage survey. Consistent with these POLA2 should be upregulated in colorectal cancer and reversibly after treatment with ETC-1922159 it was found to be down regulated. The pipeline assigns a low rank of 36 for this down regulation.

CDK5RAP2 (CDK5 regulatory subunit-associated protein 2) has been found to function in centrosome to spindle pole attachment and DNA damage response Barr *et al.*[102]. Mutations in CDK5RAP2 have caused premature depletion in neural stem cells and thus microcephaly. Its role in colorectal cancer is not much known. However, CDK5RAP2 was found to be down regulated after the drug treatment and assigned a low rank of 38 for down regulation.

Yu *et al.*[103] have elucidated the combination of various WNTs and (Frizzled) FZD in ventricular septal defects. In one of the observations, they find the combination of WNT10B and FZD7 to be very high. However,[104] did not include the WNT10B for the combinatorial study. FZD7 has been found to be highly expressed in colorectal cancer cells[105] &[106]. Ater the ETC-1922159 treatment, FZD7 was found to be highly suppressed. Further ranked confirmation in the 3^{rd} order combination of SCO1-WNT10B-FZD7 is depicted by a very low priority of 39.

SGOL1 or Shugoshin-like 1 is a protein encoded by SGOL1 gene and is a key protein that protects sister chromatids from premature separation during mitosis[107]. Kahyo *et al.*[107] found SGOL1 to be down regulated in colorectal cancer cases. SGOL1 are known to be tumor suppressor gene and found to be mutated in colorectal cancer cases also[108]. Mutations in SGOL1 or its down regulation means there is no prevention of premature separation which leads to different kinds of instability as can be found by presence of microsatellite instability in colorectal cancer cases. After treatment of ETC-1922159, SGOL1 has been found to be down regulated in treated colorectal cancer cells. This points to the fact that mutated versions of SGOL1 might have been suppressed as wild type SGOL1 would have been upregulated in cured cells. The pipeline points to this down regulation with a rank of 40.

TP73 or p73 is a tumor suppressor belonging to the family of p53 transcription factors[109]. It is known to be upregulated in colorectal cancer case[110]. Dysfunction in p73 leads to mitotic abnormalities causing polyploidy and aneuploidy which contributes to tumorigenesis[111]. Consistent with these, after the ETC-1922159 treatment, TP73/p73 was found to be down regulated. Probably the dysfunction of TP73 was suppressed after the drug administration. A low rank of 41 confirms the pipeline's indication of down regulation.

PAK4 has been found to be expressed in breast cancer cells and depletion of the same modifies cell adhesion dynamics[112]. Dart *et al.*[112] show that reduced expression of PAK4 leads to loss of RHOU and RHOU is ubiquitinated via RAB40A-CULLIN5 complex. Expression of PAK4 rescues RHOU ubiquitination. Also RHOU expression assists PAK4 expression. Thus RAB40A is often found to be underexpressed in breast cancer. Furthermore, knockdown of PAK4 inhibits proliferation of mutant KRAS colorectal cancer cases[113]. Its expression would lead to proliferation. Analogous to the breast cancer case, RAB40A might be underexpressed in colorectal cancer case as PAK4 helps in proliferation. However, in colorectal cancer cases mutations in RAB40A could be present which do not help in targeting RHOU degradation and thus via PARK4 and RHOU are expressed in colorectal cancer case. Following this line of thought, ETC-1922159 administration lead to down regulation of RAB40A. It might be that the mutated versions of RAB40A have been suppressed after the drug treatment. The pipeline indicates the low rank of 42 apropos this down regulation.

TGIF2 is found to be expressed in colorectal cancer case[114] and is actually a transcriptional repressor that works by recruitment of HDAC3 (histone deacetylase 3). After administration of ETC-1922159, TGIF2 was found to be down regulated and this down regulation was assigned a value of 43.

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PDRG1 is a novel p53 and DNA damage-regulated gene that has been found to be up regulated in colon cancer cases and knock down of the same has shown marked slowdown in tumor growth [115]. Jiang *et al.* [115] showed that PDCD7 (programmed cell death 7) has been found to be interacting with PDRG1 and implicates PDRG1 in cell growth regulation via involvement in apoptosis and cell cycle regulation. After the treatment of ETC-1922159 drug PDCD7 was found to be down regulated. This down regulation indicates the inactivation of PDRG1 which thereby slows down tumor growth. The pipeline points to this down regulation via a low rank of 45.

Glutathione peroxidase 1 or GPX1 is an antioxidant enzyme that helps in protecting cells from oxidative stress via reduction of hydrogen peroxide to H_2O. Polymorphisms of the gene have been related to increased risk in cancer [116] and Goldberg *et al.* [117] show that GPX1 has a loss of heterozygosity at later stages of colon carcinogenesis. Consistent with these GPX1 was found to be down regulated after the ETC-1922159 drug, indicating that the drug might be restricting the process of oxidative stress by suppressing the polymorphed GPX1. The pipeline assigns this down regulation with a value of 47.

Kinesin superfamily (KIF) members share a highly conserved protein family and are known to be involved in motor binding as well as transportation of vesicles and organelles [118]. Liu *et al.* [118] show that KIF20B is known to be overexpressed in colorectal cancer case. After the ETC-1922159 administration KIF20B is found to be down regulated and the pipeline points to this observation by assigning a low rank of 49.

LAT2 (Large neutral amino acids transporter small subunit 2) is a family of LAT proteins that is encoded by SLC7A8 gene. These are Na^+-independent transporters that deliver neutral amino acids into cells and have been responsible for cellular leucine uptake, protein translation and cell growth [119]. The LAT2 is found to be expressed in colorectal cancer and is regulated by the expression of MYC [120]. Satoh *et al.* [120] show that the knockdown of the MYC that is involved in metabolic reprogramming, lead to decrease in the levels LAT2 (SLC7A8). SLC7A8 was found to be down regulated in colorectal cancer cells treated with ETC-1922159 and the pipeline points to this down regulation with a low rank of 52.

The role of IL-17 (Interleukin-17) family is known to be controversial in CRC, however there are cases were it has been reported to be a prognostic marker for colorectal cancer Lin *et al.* [121] & [122]. A homologue of the family, IL-17D a novel cytokine has been discovered [123] and found to play a role in many of the cancers. In cells treated with ETC-1922159, IL-17D was found to be down regulated and reversibly it must have been regulated in the colorectal cancer cases. A low ranking of 55 along with WNT10B-SCO1 relates to the down regulation after the drug treatment.

ING5 (Inhibitor growth protein 5) has a controversial role and sometime it is found to work as a tumor suppressor by binding to p53 and enhancing p53 activity [124] while at other times it has been reported to play a role of oncogene at cytoplasmic level but not at nuclear level [125]. Tallen and Riabowol [126] claim overexpression of ING5 in colorectal cancer cases. After treatment with ETC-1922159, ING5 was found to be down regulated and in context of the WNT10B-SCO1 combination, the pipeline indicates the suppression with a rank of 56. Probably, as Zheng *et al.* [125] suggest, ING5 is up regulated at cytoplasmic level in colorectal cancer cases.

MNS1 (meiosis specific nuclear structural 1) was found to be down regulated via knockdown of KLK6 [127]. KLK6 is observed to be highly regulated in colorectal cancer cases and facilitates in the invasion-metastasis formation via specific downstream network of miRNA-mRNA effectors. Furthermore, oxaliplatin treatment lead to down regulation of MNS1 in colorectal cancer cell lines [128]. Not much is known about MNS1 in colorectal cancer case and after treatment with ETC-1922159 in colorectal cancer cells, it was found to be down regulated. This down regulation is assigned a rank of 57. Further investigations are needed for MNS1 with respect to WNT10B and SCO1.

DDN or dendrin is a neural protein that is usually found to be functional in brain and kidney. The authors are unclear how and why DDN was chosen for study after the administration of ETC-1922159 in [51]. However, DDN was found to be down regulated after the administration of the drug and the pipeline assigned a ranking of 61 for the downregulation.

CBX5(Chromobox Protein Homolog 5) also known as heterochromatin protein 1α or HP1α in humans is known to act as a gene silencer [129]. Unphosphorylated STAT5 is a tumor suppressor that inhibits multiple oncogenes by binding to CBX5/HP1α to stabilize heterochromatin [130]. This formation of heterochromatin and involvement of epigenetics leads to tight packing of the genes and consecutive folding of DNA such that transcription of oncogenes is inhibited. Reduction in HP1α/CBX5 levels have been found to instigate cancer progression [131]. CBX5 was found to the down regulated in colorectal cancer cells after the treatment of ETC-1922159 [51]. Mechanistically, over expression of CBX5 should suppress the tumor progression. This points to the fact that mutations/defects in CBX5 might have been present in colorectal cancer cases which could not help in stabilization of heterochromatin and the administration of the drug lead to its down regulation. This down regulation is indicated by a low rank of 64 by the pipeline.

C4orf46 (Chromosome 4 open reading frame 46) was found to be down regulated after the administration of the drug ETC-1922159. Not much is known about the role of C4orf46 in colorectal cancer. The pipeline indicates a down regulation with a low rank assignement of 69.

FAM131B (Family with sequence similarity 31 member B) has

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been found make fusions with BRAF and is involved in some of the cancers like pilocytic astrocytoma[132]. Oncogenic fusions like FAM131B-BRAF are found mostly in brain tumors. Not much is known about FAM131B in colorectal cancer and administration of drug in experiments[51] showed that FAM131B is down regulated in treated colorectal cancer cells. Consistent with these, the pipeline points to this down regulation with a rank of 70. Family with sequence similarity 168 member A (FAM168A) was found to under go somatic mutations in colorectal cancer case[133]. A low rank of 191 was allocated by our pipeline and indicates to the observed down regulation after administration of ETC-1922159.

RRM1 (Ribonucleoside-diphosphate reductase large subunit) [134] helps in the formation of deoxyribonucleotides prior to DNA synthesis. The role of RRM1 is also known from its association with PABC1L (see earlier discussion in the section of SCO1-WNT10B-X combinations). PABPC1L (as recorded by[51]) or the cytoplasmic 1-like poly(A)-binding protein[60], belong to a family of multifunctional proteins PABPs which regulate and stabilize the mRNA translation. They are observed to be helpful in the transportation of the mRNA also from the nucleus and there exists a nucleic version of PABP also[61]. PABPC1 contains four non identical RRMs that are joined with the main PABC domain and separated by a linker. We know that PABC family is down regulated after the treatment of ETC-1922159 and expect that RRM1 should also be down regulated after the drug administration. This is confirmed by experiments in[51]. Additionally, wild type RRM1 is known to be highly expressed in colorectal cancer[135] &[136]. Consistent with these, after the treatment of ETC-1922159, RRM1 was found to be down regulated and the pipeline points to this via a low rank of 71 along with SCO1-WNT10B.

Long non-coding RNA facilitate in protein coding and non coding and recently, aberrations in the same have been found to promote various cancers. FOXD2-AS1 has been implicated in gastric cancer[137] as well as the non-small lung cancer[138]. FOXD2-AS1 has recently been found to be expressed in colorectal cancer and promotes the same via regulating EMT and Notch signaling pathway[139]. Consistent with the recent finding and the experiments of ETC-1922159 drug administration[51], FOXD2-AS1 was found to be down regulated after the treatment in colorectal cancer cells. A low rank of 72 was assigned to FOXD2-AS1 along with WNT10B and SCO1. Further investigation for FOXD2-AS1 is needed at in vitro/in vivo level.

MARVELD1 (MARVEL domain containing protein 1 and unfortunately not the MARVEL comics) has been found to bind to the importin-β1 (IPOβ1, a kind of nucleocytoplasmic protein that helps in transportation of proteins between the nucleus and the cytoplasm). It has been found to be MARVELD1 shows decreased expression in tumor cases and binds to IPOβ1[140]. However, the functionality of MARVELD1 in colorectal cancer is not known and Madan *et al.*[51] report the down regulation of the same after the administration of ETC-1922159 drug. The pipeline assigns a rank of 75 for this reported down regulation.

NUBPL (or the nucleotide-binding protein-like) encodes the iron-sulfur (Fe/S) protein (IND1) and has a role in the assembly of mitochondrial complex 1[141]. Mitochondrial complex 1 is a member of the mitochondrial respiratory chain. Recently, NUPBL has been found to be highly expressed in colorectal cancer cases and Wang *et al.*[142] show that this is due to the induced affect of NUPBL expression in EMT. EMT is a major process which helps in metastasis in cancers. Consistent with these, NUBPL was found to be down regulated after the treatment of ETC-1922159. This down regulation is assigned a low rank of 78 which is in line with the findings in[51].

TAMM41 or mitochondrial translocator assembly and maintenance protein was found to be down regulated after treatment of ETC-1922159. Currently, the authors are not much aware of the affects of TAMM41 in colorectal cancer cases, however, the reversible picture is that TAMM41 is highly regulated in colorectal cancer cell and after the administration of the drug the down regulation was assigned a value of 80.

ARHGAP11B has been found to be highly expressed in the development of mouse and human neocortex[143]. However, its role in colorectal cancer is not known explicitly. Madan *et al.*[51] report the down regulation of this gene after the administration of ETC-1922159. We do not know why this is so, however the pipeline indicates this down regulation with a low rank of 85.

RRS1 (Ribosome biogenesis regulatory protein homolog) is been found to be a highly conserved gene, deletions/mutations of which lead to transcription repression of ribosomal protein[144]. It has also been found to up regulated in colorectal cancer[145],[146] &[147], indicating the expression of ribosomal proteins which might be effective in tumor. Consistent with these, RRS1 was down regulated after the treatment of ETC-1922159 and the pipeline pointed to the down regulation with a low rank of 89 along with SCO1-WNT10B combination.

PPT1 (Palmitoyl-protein thioesterase 1) is a small glycoprotein involved in the catabolism of lipid modified proteins during lysosomal degradation. Defects in these genes have been implicated in infantile neuronal ceroid lipofuscinosis[148] &[149]. Tsukamoto *et al.*[150] observe overexpression of CLN1 in colorectal cancer which encodes PPT for removal of fatty acids from fatty-acylated cysteineresidues in proteins[148]. Consistent with these, ETC-1922159 treatment of colorectal cancer cells lead to the the down regulation of PPT1 and the pipeline indicates this with a low rank of 91.

Cortactin-binding protein 2 is encoded by CTTNBP2 and has been found to be up regulated in APC driven tumorigenesis Gaspar *et al.*[151]. Tuupanen *et al.*[152] observe mutations in CTTNBP2 in colorectal cancer cases. Nehrt *et al.*[153] also somatic mutations in colon cancer. After the treatment of ETC-1922159, CT-

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TNBP2 was found to be down regulated and this is indicated by the pipeline with a low rank of 93.

MEGF8 encoded Multiple Epidermal Growth Factor-like Domains 8, is a single pass membrane protein that facilitates cell communication and developmental regulation[154]. MEGF8 has been found to be significantly associated with colorectal cancer cases in[155]. Mechanistic role of MEGF8 has not been explored much in colorectal cancer and after the administration of the drug, it was found to be down regulated. The pipeline indicates this down regulation with a low rank of 97.

Hydroxyacyl-Coenzyme A dehydrogenase is encoded by gene HADH and mutations in the same have been found to cause hyperinsulinemic hypoglycemia[156]. Deficiency in HADH can lead to a rare condition where body stops converting fat into energy. The authors are not aware of HADH in colorectal cancer and the pipeline indicated a low rank of 107 along with SCO1-WNT10B. After the administration of the drug, HADH was found to be down regulated in the treated colorectal cancer cells with ETC-1922159.

ATAD3A (ATPase family AAA domain containing 3A) was found to interact with WASF3 which is a metastasis promoting gene[157]. ATAD3A is a mitochondrial membrane protein and Teng *et al.*[157] show that knockdown of ATAD3A lead to decreased levels of WASF3. Furthermore, silencing of ATAD3A causes loss of invasion and suppression of tumor growth. Consistent with these, administration of ETC-1922159 lead of down regulation of ATAD3A and the pipeline assigns a low rank of 110.

Mitochondrial Intermembrane Chaperone TIMM9 has been found to be over expressed in gastric cancer cases[158]. Encoded protein are involved in the transportation of the membrane proteins into the mitochondrial inner membrane. TIMM9 was found to be up regulated in colorectal cancer case[145]. Consistent with these, our pipeline indicated the down regulation of TIMM9 by ETC-1922159[51], with an assignment of low rank of 124.

TARS2 has been found to be implicated in epilipsey[159]. Its role in colorectal cancer is not much known and after ETC-1922159 treatment TARS2 was found to be down regulated. This down regulation is pointed to with a rank of 133 by the pipeline.

MTHFD2L (NAD-dependent methylenetetrahydrofolate dehydrogenase 2-like protein)[160] functions within the inner mitochondrial membrane. MTHFD2L is a part of mitochondrial pathway and facilitates in the conversion of folate to formate. Mitochondrial folate-coupled metabolism plays role in cell proliferation and MTHFD2L has been found to be highly expressed in many tumors[161] &[162]. Consistent with these MTHFD2L was found to be expressed in colorectal cancer cells and after the administration of ETC-1922159 it was down regulated[51]. Our pipeline allocates a low rank of 136 for this down regulation. Similarly, another variant MTHFD1L was allotted a low rank of 157.

We earlier saw the role of PDCD7 were[115] showed that PDCD7 (programmed cell death 7) has been found to be interacting with PDRG1 and implicates PDRG1 in cell growth regulation via involvement in apoptosis and cell cycle regulation. PDRG1 has been found to be up regulated in colon cancer cases. A variant of PDCD7, i.e. PDCD4, also inhibits migration and invasion in colorectal cancer. Silencing of HNRNPC lead to the inhibition of migration and invasion in T98G cells, thus supporting the fact that HNRNPC regulates invasion and metastasis via regulation of PDCD4[163]. HNRNPC (Heterogeneous nuclear ribonucleoproteins C1/C2)[164] is usually found to be expressed in fetuses and lead to birth defects Zhang *et al.*[165]. Nevertheless, after the ETC-1922159 treatment, HNRNPC was found to be down regulated and the pipeline assigned a low rank of 137.

Overexpression of GINS has been found in colorectal cancer [166]. Furthermore, PSF3 which is a component of the tetrameric complex GINS, has a major role in colon cacner cell proliferation [167]. Consistent with these, ETC-1922159 administration lead to GINS3 suppression[51] and our pipeline allocated a low rank of 149.

METTL16 (methyltransferase-like protein 16) is known to bind with metastasis associated lung adenocarcinoma transcript 1 MALAT1 which is a cancer promoting long noncoding RNA. This binding happens via a triple RNA binding helix element as Brown *et al.*[168] have observed. MALAT1 is a long non coding RNA whose 3' functional motiff plays a role in the cell proliferation, invasion and metastasis in CRC[169]. Yeon *et al.*[170] show frame shift mutations in METTL16 in cases of colon cancer. Consistent with these, ETC-1922159 induced inhibition in cancer growth and METTL16 was found to be down regulated. Probably, MALAT1 must also have been down regulated as it works in combination with METTL16. This needs to be verified in vitro/in vivo. Our pipeline also indicated a low rank for this down regulation with a rank of 151. A variant METTL12[171] was also found to be down regulated and the pipeline assigned a low rank of 174. However, how METTL12 plays a role in colorectal cancer needs to be investigated and research is ongoing.

SEC31 is a protein in yeasts essential for endoplasmic recticulum-golgi body transport[172]. Its homologue SEC31A and SEC31B are prevalent in humans. In human intestinal epithelial cells, SEC31 depletion was shown to causes defective epithelial polarity and organization on permeable supports[173]. Sec13-Sec31 heterotetramer[174] is thought to link with a pre-budding complex and drive the membrane deformation to form COPII vesicles[175]. SEC24C is an essential component of COPII and a potential marker for colorectal cancer[176]. Consistent with these, SEC31B was found to be down regulated after ETC-1922159 and our pipeline points to this with a low rank of 159.

Similar to role of SCO1 in the respiratory chain reactions in mitochondria, BOLA3 has been found to be play a role in mitochondria. Iron-sulfur (Fe-S) clusters in the mitochondria have

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been found to play crucial role in many of the cellular processes [177]. Genes NFU1 and BOLA3 (and encoded proteins) facilitate in the formation of complexes along with other factors that help in the biogenesis and stabilization of the Fe-S centers for assembly of respiratory chain complexes in mitochondria and normal maturation of lipoate-containing 2-oxoacid dehydrogenases [178], among the various other processes. Mutations in NFU1 and BOLA3 have been found to cause genetic diseases with defects in mitochondrial Fe-S centers [179]. Expression of BOLA3 was found to be significantly altered in colorectal cancer cases [180] and down regulated after the ETC-1922159 treatment in colorectal cancer cells [51] and our pipeline indicates this down regulation with a low rank of 188.

Tubulin alpha-1B chain (TUBA1B) was found to be significantly expressed in colorectal cancer cases [181]. MKI67 and TUBA1B were found to be expressed in cycling LGR5$^+$ intestinal stem cells [182]. LGR-4/5/6 is known to work RNF families to inhibit the FZD families and thus inhibit the Wnt signaling. Along with RSPO, the signaling is up regulated as LGR-RNF go through degradation process. TUBA1B was found to be down regulated after the administration of ETC-1922159 and our pipeline assigned the down regulation with a low rank of 202.

ACTL6A (Actin-like protein 6A) was found to be up regulated in HCC and play major role in metastasis and EMT of HCC [183]. It has been found to be co-amplified with p63 in squamous cell carcinoma and is a poor proginator [184]. Also, ARID1A normally targets SWI/SNF complexes and acts a tumor suppressor in colon cancer [185]. Finally, ACTL6A prevents SWI/SNF chromatin-remodelling complexes to regulate many of the differentiation genes to maintain epidermal progenitor state [186]. It might be that in colorectal cancer case ACTL6A is highly active and prevents the prevents SWI/SNF for regulation of oncogenes. This needs verification, however, ACTL6A was found to be down regulated after the treatment of ETC-1922159 and this down regulation was allotted a low rank of 216.

GC-rich sequence DNA-binding factor (GCFC2) factor were found to be differentially expressed in celecoxib treated hereditary nonpolyposis colon cancer patient cells [187]. GCFC2 was also found to be down regulated after the treatment of ETC-1922159 in colorectal cancer cells [51]. Our pipeline assigned a low rank of 226 regarding this down regulation.

Interferon regulatory factor 8 (IRF8) is a transcription factor that promotes regulation of lineage commitment. It has been known to have an inverse relation with colorectal cancer metastasis [188] via promotion of apoptosis and had been found to be suppressed in colorectal cancer cases. Deficiency in IRF8 promotes inflammation-mediated colon tumorigenesis [189]. Given this case, mutations in IRF8 could lead to tumorigenesis and administration of ETC-1922159 might have caused the inhibition of tumor growth where mutations IRF8 would have been present. Our pipeline suggests the down regulation of probable mutated IRF8 in treated colorectal cancer cells with a low rank of 227.

PAX-interacting protein 1 (PAXIP1-AS2) is known to play role in genomic stability and chromatin condensation, and has been found to play a role in colorectal cancer case [190]. After treatment of ETC-1922159, PAXIP1-AS2 was found to be down regulated in colorectal cancer and our pipeline assigned a low rank of 228.

Nucleoporin 160 (NUP160) [191] is one of the proteins that make up for the nuclear pore complex [192] which helps in nucleoplasmic transport. In a proteomics approach Albrethsen *et al.* [193] report down regulation of NPC which involves NUP160, indicating cellular and nuclear crisis. Mutations in NUP160 and overall NPC might be a play a role in colon cancer. However, Shitashige *et al.* [194] show that NPC plays major role in regulating Wnt pathway. Consistent with these, our pipeline assigned a low rank of 236 for the down regulation of NUP160 in colorectal cancer cells treated with ETC-1922159.

CD3EAP encodes DNA-directed RNA polymerase I subunit RPA34. CD3EAP (CD3e antigen, epsilon polypeptide associated protein) is also known by the name of ASE-1 (Anti Sense ERCC1) [195] increased polymorphisms of which have been associated with increased risk of colorectal adenomas and carcinoma in a Norwegian cohort [196]. However, in a Danish study CD3EAP was not found to play any role in colorectal cancer [197]. CD3EAP polymorphisms has been found to be associated in chronic atrophic gastritis also [198]. After administration of ETC-1922159 CD3EAP was found to be down regulated and our pipeline assigned this down regulation of CD3EAP with a low rank of 256. However, not much research work has been done on SNP variations of CD3EAP in colorectal cancer case.

XPOT encodes protein exportin-t, a necessary component that is used for the export of tRNA from the nucleus to the cytoplasm via GTP-bound RAN [199] & [200]. We earlier saw that CRM1 (chromosomal region maintenance 1 also XPO1/exportin 1) has been found to play a major role in the export process from nucleus to the cytoplasm while dealing with PABPC1L. The crystal structure of CRM1 suggests binding with RAN protein along with GTP, allowing for a conformational change that facilitates binding to different cargo proteins through a nuclear export signal (NES) [201] & [202]. Inhibition of CRM1 pathway has been found to arrest the transport of various oncoproteins and retention of various tumor suppressor factors [203] & [204]. XPOT work in a similar fashion as CRM1 in binding with RAN-GTP for the export of mature tRNAs [199]. In colorectal cancer cases (MSI), XPOT was found to be mutated [205]. After the treatment of colorectal cancer cells by ETC-1922159 [51], XPOT was found to be down regulated and our pipeline shows assigns this down regulation with a low rank of 276. Probably, the colorectal cancer cases contain mutated versions of XPOT that might lead to transfer of oncoproteins and ETC-1922159 might act as an inhibitor for mutated XPOT.

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SNHG16 (snoRNA host gene 16) has been demonstrated to be significantly up regulated in adenomas and all stages of CRC [206]. Christensen *et al.* [206] report positive correlation with Wnt regulated factors like ASCL2 which is known for contributing to stemness. Also, silencing of SNHG16 has been found to affect lipid metabolism and increase apoptotic cell death. Based on these, after the ETC-1922159 treatment in colorectal cancer cells, SNHG16 was found to be down regulated and our pipeline allocates the same with a rank of 280.

Transforming growth factor β 1 or TGFβ1 [207] is encoded by TGFB1 and found to play multiple roles in processes like cell proliferation, growth, differentiation and apoptosis. It is known to be the most abundant isoform of TGFβ family and has been found to be highly expressed in colorectal cancer case [208], [209] & [210]. Consistent with these findings, TGFB1 was found to be down regulated after the ETC-1922159 treatment of colorectal cancer cells [51]. In silico, our pipeline indicated the down regulation with a rank of 298.

TBGR4 or Transforming growth factor beta regulator 4 is a part of FASTKD family of proteins that is involved in regulating the energy balance of mitochondria under stress and cell cycle progression [211]. TBGR4 has been found to be implicated in colorectal cancer [212]. After ETC-1922159 treatment [51], TBGR4 was down regulated and our pipeline indicated this with a low rank of 336.

DNASE2 (Deoxyribonuclease II, lysosomal) is known for engaging in the break down of DNA during apoptosis [213]. DNASE2 was found to be down regulated after the treatment of ETC-1922159 in colorectal cancer cells and our pipeline points to this with a rank of 354. Not much is known about the role of CAAP1 (Caspase activity and apoptosis inhibitor 1) in colorectal cancer and it was found to be down regulated after the treatment of ETC-1922159 [51]. Our pipeline assigned a low rank of 410. ELAC2 (Zinc phosphodiesterase ELAC protein 2) is involved in the maturation of tRNA within the mitochondria. ELAC2 has been found to play a role in prostate cancer [214], while its role in colorectal cancer is still ongoing. After the administration of ETC-1922159 drug, ELAC2 was found to be down regulated and our pipeline shows this down regulation with a low rank of 424.

SMC1A (Structural maintenance of chromosomes 1A) belongs to the family of the SMC proteins that are used for the cohesion of the sister chromatids [215] & [216]. Over expression of SMC1A has been found to be a poor prognostic marker in colorectal cancer cases [217] & [218]. SMC1A is known to recruit TAF (tumor associated fibroblasts) for promotions of invasiveness and formation of fibroblasts which assist in tumorigenesis [219]. Consistent with these, our pipeline assigned a low rank of 438 to the observed [51] down regulation of SCM1A after the administration of ETC-1922159.

Selenium is known to be anticarcinogenic in nature and has been found to prevent cancer via the Selenium binding proteins [220] & [221]. Selenium-binding protein 1 is encoded by SELENBP1. In colorectal cancer cases, SELENBP1 has been found to be down regulated [222]. Given the above scenario, administration of ETC-1922159 showed down regulation of SELENBP1 [51] and our pipeline assigns a relatively low rank of 516. SELENBP1 should be down regulated in colorectal cancer cells, which not being the case, indicates mutations in SELENBP1 would have been present in CRC samples used in Madan *et al.* [51] and the administration of the drug led to down regulation of mutated SELENBP1. Or, the authors hypothesize that the ETC-1922159 drug is not effective on wild type SELENBP1 and thus the observed data on SELENBP1 might need further testing. This is due to the fact that suppression of SELENBP1 has been found to be a late event in colorectal cancer [223]. However, when we look across the ranking of the other tables the ranking of SELENBP1 has been found to be associated with a very high ranking on majority basis and this points to the fact that SELENBP1 should be up regulated to suppress the cancer cells as it is anticarcionogenic in nature and the effect of ETC-1922159 on SELENBP1 is not that potent. Finally, higer ranks also suggest that these combinations might not be of importance. Further chemical analysis might reveal information about ETC-1922159 on SELENBP1.

Reduced HUGL1, a homologue of LGL tumor suppressor, is found to contribute to progression of colorectal cancer [224]. LGL has been found to arrest G1 cell cycle via formation of a complex involving LGL-VPRBP-DDB1 [225] & [226]. Yamashita *et al.* [225] show that depletion of depletion of VPRBP leads to rescue of over proliferation of LGL-depleted cells. Mutations in VPRBP might lead to cell proliferation in colorectal cancer cases and ETC-1922159 administration show down regulation of VPRBP. Reversibly, down regulation of wild type VPRBP leads to phase progression. Our pipeline shows a down regulation with a rank of 656 for the mutated version, however, across different tables, the majority voting points to higher rank. This high rank indicates the wild type VPRBP to be work reversibly and thus point to inhibition of progression of cell proliferation. Also, higher ranks also mean that these combinations might not be important in one specific condition while it might be in another. Further tests are needed for VPRBP.

Carbonyl reductase 1 is encoded by CBR1 gene [227]. It has been found to show protective role against cellular damage from oxidative stress and apoptosis [228] and [229]. It has been found to be highly regulated in colorectal cancer cases and known to build Doxorubicin resistance in human gastrointestinal cancers [230]. Consistent with these, CBR1 was found to be down regulated after the ETC-1922159 treatment and our pipeline shows indicates this with a low rank of 1918.

Lysyl-tRNA synthetase is an enzyme that is encoded by KARS. Mutations have been found in KARS in colorectal cancer cases [231]. However, our pipeline showed a high rank for SCO1-WNT10-KARS with an assignment of 2793. This indicates that the combi-

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nation might not be of value after the treatment of ETC-1922159. Combination of KARS with other components showed consistent behaviour and was found to be down regulated and assigned proper rank (see other tables).

ADP-ribosylation factor-like protein 9 or ARL9 belongs to the family of ARL[232] and not much has been studied regarding it's role in colorectal cancer. However, it was found to be down regulated after the ETC-1922159 treatment and our pipeline indicates a high rank of 3196 along with SCO1-WNT10B. This indicates that this combination might not be useful for investigation. Nevertheless, biologists might want to confirm negative results also in wet lab.

ODF2 or Outer dense fibre protein 2 has been implicated in fertility[233]. Not much research work as been found in context of the role of ODF2 in colorectal cancer case[234], however, for the combination with SCO1-WNT10B, the pipeline showed a high rank of 4512, indicating not much importance. Also, other combinations (see other tables) show similar ranking with not much importance.

PDE7A encodes high affinity cAMP-specific 3',5'-cyclic phosphodiesterase 7A[235] and is found to be highly expressed in colorectal cancer case[236]. Contrary to this, our pipeline assigned a high rank of 4728, indicating that this combination with SCO1-WNT10B is of not much significance after the ETC-1922159 treatment. However, the rankings of the other combinations of the PDE7A are consistent with the down regulation of ETC-1922159 treatment (see the other tables).

Centrosomal protein of 78 kDa or CEP78 is found to be a tumor suppressor and low expression of the same is associated with poor prognosis of colorectal cancer patients[237]. Also, note that CEP78 controls centrosome homeostasis by inhibiting VPRBP associated complex[238]. It was found to be down regulated after the treatment of ETC-1922159. Probably the mutated versions of CEP78 might have been present in the colorectal cancer cells, before the treatment. Also, the pipeline assigns a very high rank (4844) and thus indicates the non significance of the perhaps mutated CEP78 role. On the other hand, low rankings of CEP78 have been found to be consistent with down regulation of other dual combinations (see other tables). This might indicate that the mutated versions of CEP78 might be playing essential role in colorectal cancer and do get sown regulated on treatment with drug.

RETNLB or resistin-like-β has been found to highly expressed in colorectal cancer[239], however, down regulation of the same after ETC-15922159 was assigned a very high rank of 4850 along with WNT10B-SCO1 combination by our pipeline. This indicates to the non significance of the combination which biologist might want to overlook.

FZD7-WNT10B-X combinations

Hitherto, we observed the behaviour of the different genes in context of the dual combination of WNT10B-SCO1. We shift our attention to another important combination and see how the respective genes are behaving in context of the dual combination WNT10B-FZD7. FZD7 has been found to be highly expressed in colorectal cancer cells[105] & [106]. Ater the ETC-1922159 treatment, FZD7 was found to be highly suppressed. Further ranked confirmation in the 3^{rd} order combination of SCO1-WNT10B-FZD7 is depicted by a very low priority of 39 in table 2 and it correlates to the ranking in table 1.

Contrary to this, most the 3^{rd} order combinations of the different genes listed in table 1 with WNT10B-SCO1 showed opposite ranking behaviour to that with WNT10B-FZD7 as shown in table 2. Many of these combinations are now ranked extremely high along with FZD7-WNT10B. FZD7-WNT10B combination is itself found to be upregulated in colorectral cancer cases and both were down regulated after the treatment of ETC-1922159. Interestingly the 3^{rd} order combinations were found to show very high ranks indicating that these would be highly regulated after the drug treatment, which might not be true. These high ranks point to the fact that the combination of the genes with WNT10B-FZD7 are not of importance after the drug treatment as the low ranked combinations WNT10B-SCO1-X. The reversal of ranks with WNT10B-FZD7 for many of the genes show that the pipeline is pointing to the ineffectiveness of the combination after the ETC-1922159 drug treatment. These combinations might not be of interest (i.e WNT10B-FZD7-X) as the X genes associated with WNT10B-SCO1 have been found to be down regulated and the pipeline assigned low ranks to them.

The assignment of high ranks by the pipeline recommend the biologists to safely ignore these combinations. Note that many of these rankings are ≥ 2425 (i.e $\frac{1}{2} \times 4850$ 3^{rd} order combinations) which point to the non significance of the combinations. Those that have ranks ≤ 2425 are of value and the biologists might want to have a look at these WNT10B-FZD7-X combinations what have been found to be down regulated after the ETC-1922159 treatment. Finally, note that these rankings are not a hard and fast rule and give a guideline to the biologists of what might be of significance. Combinations lying on the border line (near to 2425) can also be tested. Also, it is not that each and every combination will have an exact reversal. In some cases there will be different behaviour and the biologists might want to tally the rankings across the tables also. For example WNT10B-SCO1-ODF2 and WNT10B-FZD10-ODF2 are of no importance due to high ranks but the combination WNT10B-ODF2-RRM1 and WNT10-B-ODF2-XX172 are of significance (see tables 3 and 4).

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RANKING USING HSIC - RBF KERNEL W.R.T WNT10B-FZD7

3^{nd} odr comb.	rbf rank	3^{nd} odr comb.	rbf rank	3^{nd} odr comb.	rbf rank
SCO1-WNT10B-FZD7	39	SMC1A-WNT10B-FZD7	126	GINS3-WNT10B-FZD7	190
TUBA1B-WNT10B-FZD7	286	XPOT-WNT10B-FZD7	356	HADH-WNT10B-FZD7	394
ARL9-WNT10B-FZD7	560	WNT10B-FZD7-PAXIP1.AS2	597	WNT10B-FZD7-PDCD7	723
WNT10B-CD3EAP-FZD7	732	WNT10B-CBX5-FZD7	818	WNT10B-MNS1-FZD7	911
GCFC2-WNT10B-FZD7	982	WNT10B-FZD7-KARS	1043	WNT10B-RRM1-FZD7	1148
WNT10B-FAM131B-FZD7	1207	WNT10B-ARHGAP11B-FZD7	1231	WNT10B-FZD7-CBR1	1337
TIMM9-WNT10B-FZD7	1510	WNT10B-FZD7-ZNF502	1567	WNT10B-IRF8-FZD7	1621
CTTNBP2-WNT10B-FZD7	1657	WNT10B-XX134-FZD7	1660	WNT10B-FZD7-FOXD2.AS1	1662
WNT10B-FZD7-CAAP1	1682	FAM168A-WNT10B-FZD7	1773	WNT10B-FZD7-ZNF594	1784
PDE7A-WNT10B-FZD7	1881	WNT10B-FZD7-TARS2	1925	WNT10B-SGOL1-FZD7	1979
WNT10B-UHRF1-FZD7	2032	WNT10B-FZD7-RRS1	2155	WNT10B-XX91-FZD7	2241
WNT10B-FZD7-NUBPL	2338	WNT10B-GFRA3-FZD7	2343	WNT10B-FZD7-NUP160	2361
WNT10B-BOLA3-FZD7	2382	WNT10B-FZD7-SELENBP1	2384	WNT10B-FZD7-C4orf46	2400
WNT10B-METTL16-FZD7	2420	WNT10B-FZD7-XX81	2540	WNT10B-SNHG16-FZD7	2586
WNT10B-PABPC1L-FZD7	2702	WNT10B-PPT1-FZD7	2789	XX16-WNT10B-FZD7	2831
WNT10B-SLC7A8-FZD7	2867	WNT10B-EXOSC5-FZD7	2958	WNT10B-LCTL-FZD7	2992
WNT10B-FZD7-SEC31B	3004	WNT10B-GPX1-FZD7	3036	WNT10B-FZD7-MARVELD1	3080
CEP78-WNT10B-FZD7	3100	WNT10B-FZD7-HNRNPC	3113	WNT10B-TXLNG-FZD7	3142
WNT10B-FZD7-XX172	3144	WNT10B-XX148-FZD7	3258	WNT10B-RCN2-FZD7	3260
RETNLB-WNT10B-FZD7	3302	WNT10B-KIF20B-FZD7	3345	WNT10B-ZNF572-FZD7	3386
WNT10B-FZD7-WDR76	3388	WNT10B-FZD7-IPO11	3406	WNT10B-FZD7-DEPDC1B	3459
WNT10B-DEPDC7-FZD7	3613	WNT10B-FZD7-ACTL6A	3678	WNT10B-ZNF740-FZD7	3694
WNT10B-EXOSC3-FZD7	3698	WNT10B-ING5-FZD7	3730	WNT10B-XX228-FZD7	3752
WNT10B-MEGF8-FZD7	3785	WNT10B-TBRG4-FZD7	3791	WNT10B-FZD7-CDCA2	3797
ODF2-WNT10B-FZD7	3816	WNT10B-FZD7-ACVR1C	3848	WNT10B-FZD7-IL17D	3863
WNT10B-DHX9-FZD7	3884	WNT10B-TGIF2-FZD7	3929	WNT10B-FZD7-TAMM41	3935
WNT10B-VPRBP-FZD7	3956	WNT10B-IPO9-FZD7	3993	WNT10B-FZD7-TTC26	4006
WNT10B-MTHFD1L-FZD7	4013	WNT10B-FZD7-DNASE2	4038	WNT10B-POLA2-FZD7	4072
WNT10B-FZD7-DDN	4091	WNT10B-FZD7-LPAR6	4100	WNT10B-CDK5RAP2-FZD7	4102
WNT10B-METTL12-FZD7	4118	WNT10B-FZD7-TP73	4125	WNT10B-ATAD3A-FZD7	4140
WNT10B-ABCA2-FZD7	4188	WNT10B-FZD7-RAB40A	4194	WNT10B-FZD7-COLEC11	4359
WNT10B-TGFB1-FZD7	4442	WNT10B-MTHFD2L-FZD7	4535	WNT10B-ELAC2-FZD7	4640
WNT10B-FZD7-LEFTY1	4651	WNT10B-FZD7-TFB2M	4707		

Table 2 3^{rd} order interaction ranking using HSIC for radial basis function kernel. Total number of 3^{rd} order interactions in a set of 100 genes - 161700. 4851 3^{rd} order combinations for WNT10B associated work. Rankings for FDZ7-WNT10B-X have been tabulated.

RRM1-WNT10B-X combinations

Earlier, we observed the behaviour of RRM1, while explaining its 3^{rd} order combination with SCO1-WNT10B. To reiterate, RRM1 (Ribonucleoside-diphosphate reductase large subunit) [134] helps in the formation of deoxyribonucleotides prior to DNA synthesis. The role of RRM1 is also known from its association with PABC1L (see earlier discussion in the section of SCO1-WNT10B-X combinations). PABPC1L (as recorded by [51]) or the cytoplasmic 1-like poly(A)-binding protein [60], belong to a family of multifunctional proteins PABPs which regulate and stabilize the mRNA translation. They are observed to be helpful in the transportation of the mRNA also from the nucleus and there exists a nucleic version of PABP also [61]. PABPC1 contains four non identical RRMs that are joined with the main PABC domain and separated by a linker. We know that PABC family is down regulated after the treatment of ETC-1922159 and expect that RRM1 should also be down regulated after the drug administration. This is confirmed by experiments in [51]. Additionally, wild type RRM1 is known to be highly expressed in colorectal cancer [135] & [136].

We found the ranking behaviour of many of the genes (X) along with WNT10B and RRM1 to follow a pattern similar to SCO1-WNT10B-X rankings. Again, not every combination will have ex-

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RANKING USING HSIC - RBF KERNEL W.R.T WNT10B-RRM1

3^{nd} odr comb.	rbf rank	3^{nd} odr comb.	rbf rank	3^{nd}odrcomb.	rbf rank
SCO1-WNT10B-RRM1	71	XPOT-WNT10B-RRM1	223	TUBA1B-WNT10B-RRM1	224
SMC1A-WNT10B-RRM1	230	WNT10B-SGOL1-RRM1	231	WNT10B-ARHGAP11B-RRM1	282
WNT10B-GFRA3-RRM1	318	WNT10B-MNS1-RRM1	334	WNT10B-RRM1-DEPDC1B	371
WNT10B-RRM1-ACVR1C	393	RETNLB-WNT10B-RRM1	399	GINS3-WNT10B-RRM1	407
WNT10B-FAM131B-RRM1	411	TIMM9-WNT10B-RRM1	418	GCFC2-WNT10B-RRM1	440
WNT10B-RRM1-COLEC11	529	WNT10B-XX134-RRM1	534	WNT10B-RRM1-NUBPL	540
WNT10B-RRM1-RRS1	543	WNT10B-IRF8-RRM1	561	XX16-WNT10B-RRM1	571
WNT10B-RRM1-ZNF594	613	WNT10B-RRM1-PAXIP1.AS2	626	WNT10B-CD3EAP-RRM1	652
WNT10B-RRM1-PDCD7	662	WNT10B-XX91-RRM1	667	WNT10B-CBX5-RRM1	694
WNT10B-RRM1-TTC26	709	WNT10B-RRM1-LPAR6	715	WNT10B-SNHG16-RRM1	726
WNT10B-RRM1-C4orf46	748	WNT10B-BOLA3-RRM1	794	WNT10B-SLC7A8-RRM1	821
WNT10B-RRM1-KIF20B	826	ODF2-WNT10B-RRM1	850	WNT10B-RRM1-TFB2M	876
WNT10B-RRM1-XX81	919	HADH-WNT10B-RRM1	926	WNT10B-RRM1-CDCA2	935
WNT10B-RRM1-TARS2	972	WNT10B-LCTL-RRM1	975	WNT10B-RRM1-EXOSC3	991
WNT10B-XX148-RRM1	1102	PDE7A-WNT10B-RRM1	1129	WNT10B-RRM1-FZD7	1148
WNT10B-TXLNG-RRM1	1168	WNT10B-RRM1-ING5	1216	WNT10B-RRM1-ACTL6A	1297
WNT10B-RRM1-DDN	1350	ARL9-WNT10B-RRM1	1364	WNT10B-RRM1-KARS	1465
WNT10B-RRM1-NUP160	1469	WNT10B-RRM1-CAAP1	1489	WNT10B-RRM1-PABPC1L	1516
WNT10B-RRM1-SEC31B	1517	WNT10B-RRM1-CBR1	1603	WNT10B-RRM1-XX172	1618
WNT10B-RRM1-IL17D	1667	WNT10B-RRM1-TP73	1735	WNT10B-METTL16-RRM1	1742
WNT10B-TGIF2-RRM1	1772	FAM168A-WNT10B-RRM1	1800	WNT10B-DEPDC7-RRM1	1806
WNT10B-RCN2-RRM1	1933	WNT10B-RRM1-RAB40A	1940	WNT10B-RRM1-MARVELD1	1957
WNT10B-RRM1-EXOSC5	2001	WNT10B-RRM1-IPO11	2004	WNT10B-RRM1-FOXD2.AS1	2072
WNT10B-ZNF572-RRM1	2107	CTTNBP2-WNT10B-RRM1	2220	WNT10B-RRM1-UHRF1	2222
WNT10B-RRM1-LEFTY1	2253	WNT10B-RRM1-WDR76	2392	WNT10B-RRM1-DNASE2	2395
WNT10B-MTHFD1L-RRM1	2399	WNT10B-PPT1-RRM1	2416	WNT10B-RRM1-ZNF502	2559
WNT10B-RRM1-TAMM41	2563	CEP78-WNT10B-RRM1	2581	WNT10B-DHX9-RRM1	2828
WNT10B-RRM1-SELENBP1	2866	WNT10B-ATAD3A-RRM1	2899	WNT10B-TBRG4-RRM1	3039
WNT10B-CDK5RAP2-RRM1	3259	WNT10B-POLA2-RRM1	3539	WNT10B-ZNF740-RRM1	3551
WNT10B-RRM1-HNRNPC	3616	WNT10B-MTHFD2L-RRM1	3642	WNT10B-MEGF8-RRM1	3702
WNT10B-XX228-RRM1	3755	WNT10B-VPRBP-RRM1	3833	WNT10B-ELAC2-RRM1	3975
WNT10B-GPX1-RRM1	4021	WNT10B-TGFB1-RRM1	4109	WNT10B-METTL12-RRM1	4315
WNT10B-IPO9-RRM1	4402	WNT10B-ABCA2-RRM1	4605		

Table 3 3^{rd} order interaction ranking using HSIC for radial basis function kernel. Total number of 3^{rd} order interactions in a set of 100 genes - 161700. 4851 3^{rd} order combinations for WNT10B associated work. Rankings for RRM1-WNT10B-X have been tabulated.

actly similar ranking. However, the pattern of ranking in table 3 matches similar to that of 1, except for the fact that the rankings for RRM1-WNT10B-X are more spread out in comparison to the rankings of SCO1-WNT10B-X which is more concentrated near the lowest rank of 1. We also find that a majority of the rankings for RRM1-WNT10B-X fall below 2425 (i.e $\frac{1}{2} \times 4850$ 3^{rd} order combinations) which clearly indicate the down regulation at 3^{rd} order level after the administration of the drug.

XX172-WNT10B-X combinations

Hitherto, we concentrated our attention on the combinations which contained two known factors in a 3^{rd} order combination, namely, SCO1-WNT10B-X, FZD7-WNT10B-X and RRM1-WNT10B-X. The area were the pipeline needs to be tested is the zone where we are confronted with unknown factors that have been recorded to be down regulated after the administration of ETC-1922159. We choose XX172, a down regulated component after the drug was administered and generated the rankings of XX172 along with WNT10B and a factor X (known/unknown). Remarkably, the pattern of ranking for XX172-WNT10B-X are sim-

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RANKING USING HSIC - RBF KERNEL W.R.T WNT10B-XX172

3^{nd} odr comb.	rbf rank	3^{nd} odr comb.	rbf rank	3^{nd}odrcomb.	rbf rank
SCO1-WNT10B-XX172	1	XPOT-WNT10B-XX172	86	TUBA1B-WNT10B-XX172	117
SMC1A-WNT10B-XX172	153	GCFC2-WNT10B-XX172	459	PDE7A-WNT10B-XX172	484
XX16-WNT10B-XX172	798	TIMM9-WNT10B-XX172	969	CEP78-WNT10B-XX172	1082
WNT10B-XX172-ZNF594	1162	WNT10B-MNS1-XX172	1316	WNT10B-XX172-RRS1	1426
WNT10B-ARHGAP11B-XX172	1483	CTTNBP2-WNT10B-XX172	1528	WNT10B-RRM1-XX172	1618
WNT10B-FAM131B-XX172	1658	WNT10B-XX172-TARS2	1716	WNT10B-XX172-NUBPL	1888
ODF2-WNT10B-XX172	1915	WNT10B-CD3EAP-XX172	1944	WNT10B-DEPDC1B-XX172	2064
WNT10B-GFRA3-XX172	2132	ARL9-WNT10B-XX172	2137	WNT10B-LPAR6-XX172	2173
GINS3-WNT10B-XX172	2401	FAM168A-WNT10B-XX172	2455	WNT10B-EXOSC5-XX172	2488
HADH-WNT10B-XX172	2527	WNT10B-XX172-C4orf46	2556	WNT10B-XX172-DDN	2592
RETNLB-WNT10B-XX172	2939	WNT10B-RCN2-XX172	2994	WNT10B-SGOL1-XX172	3094
WNT10B-IRF8-XX172	3105	WNT10B-FZD7-XX172	3144	WNT10B-COLEC11-XX172	3173
WNT10B-GPX1-XX172	3186	WNT10B-LCTL-XX172	3252	WNT10B-XX81-XX172	3255
WNT10B-IPO9-XX172	3256	WNT10B-CDCA2-XX172	3303	WNT10B-XX134-XX172	3317
WNT10B-ACVR1C-XX172	3329	WNT10B-SNHG16-XX172	3342	WNT10B-IL17D-XX172	3370
WNT10B-CBX5-XX172	3430	WNT10B-LEFTY1-XX172	3437	WNT10B-UHRF1-XX172	3443
WNT10B-ZNF572-XX172	3488	WNT10B-SLC7A8-XX172	3596	WNT10B-KIF20B-XX172	3599
WNT10B-XX91-XX172	3675	WNT10B-TFB2M-XX172	3799	WNT10B-PABPC1L-XX172	3826
WNT10B-ATAD3A-XX172	3836	WNT10B-PDCD7-XX172	3880	WNT10B-METTL12-XX172	3932
WNT10B-TXLNG-XX172	3940	WNT10B-METTL16-XX172	3969	WNT10B-TP73-XX172	3970
WNT10B-BOLA3-XX172	3977	WNT10B-POLA2-XX172	4005	WNT10B-XX148-XX172	4023
WNT10B-TTC26-XX172	4041	WNT10B-PAXIP1.AS2-XX172	4099	WNT10B-PPT1-XX172	4110
WNT10B-EXOSC3-XX172	4112	WNT10B-SELENBP1-XX172	4159	WNT10B-WDR76-XX172	4167
WNT10B-ING5-XX172	4208	WNT10B-XX228-XX172	4224	WNT10B-ZNF502-XX172	4234
WNT10B-VPRBP-XX172	4240	WNT10B-FOXD2.AS1-XX172	4273	WNT10B-ABCA2-XX172	4284
WNT10B-DEPDC7-XX172	4326	WNT10B-XX172-RAB40A	4337	WNT10B-DHX9-XX172	4352
WNT10B-MTHFD1L-XX172	4388	WNT10B-MARVELD1-XX172	4412	WNT10B-ACTL6A-XX172	4447
WNT10B-TBRG4-XX172	4486	WNT10B-NUP160-XX172	4502	WNT10B-TGFB1-XX172	4554
WNT10B-KARS-XX172	4606	WNT10B-MEGF8-XX172	4613	WNT10B-ZNF740-XX172	4625
WNT10B-CDK5RAP2-XX172	4627	WNT10B-TAMM41-XX172	4635	WNT10B-TGIF2-XX172	4642
WNT10B-ELAC2-XX172	4670	WNT10B-SEC31B-XX172	4685	WNT10B-MTHFD2L-XX172	4752
WNT10B-IPO11-XX172	4760	WNT10B-DNASE2-XX172	4773	WNT10B-CBR1-XX172	4775
WNT10B-CAAP1-XX172	4781	WNT10B-HNRNPC-XX172	4840		

Table 4 3^{rd} order interaction ranking using HSIC for radial basis function kernel. Total number of 3^{rd} order interactions in a set of 100 genes - 161700. 4851 3^{rd} order combinations for WNT10B associated work. Rankings for XX172-WNT10B-X have been tabulated.

ilar to FZD7-WNT10B-X. Note that many of these rankings are ≥ 2425 (i.e $\frac{1}{2} \times 4850$ 3^{rd} order combinations) which point to the non significance of the combinations. Those that have ranks ≤ 2425 are of value and the biologists might want to have a look at these WNT10B-FZD7-X combinations what have been found to be down regulated after the ETC-1922159 treatment. Table 4 shows the rankings of XX172-WNT10B with a range of 100 recorded down regulated genes after the administration of the drug.

It might be a possibility that XX172 shows similar behaviour of up regulation along with WNT10B as FZD7 in colorectal cancer case. Similar to WNT10B-FZD7 combination, the majority of the recorded genes might not be correlating with the functionality of WNT10B-XX172. Both WNT10B and FZD7 were found to be down regulated, however, their combination with the X showed very high ranking indicating that the factor X was not in synchronization with WNT10B-FZD7. Similar is the case with WNT10B-XX172 dual combination.

Conclusion

Third order combinations related to SCO1, RRM1, FZD7 and XX172, each in conjugation with WNT10B and range of 100

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down regulated genes affected after ETC-1922159 treatment have been ranked. These rankings reveal the hitherto unknown/untested/unexplored combinations in the Wnt pathway that might be playing a major role directly or indirectly in colorectal cancer case. SCO1-WNT10B-X and RRM1-WNT10B-X showed similar ranking behaviour with a majority of combinations being down regulated and assigned a low priority rank. Contrary to this, a majority of FZD7-WNT10-B-X and XX172-WNT10B-X combinations showed no synchronization after being assigned a high priority indicating up regulation, which is not the case. Similar ranking pattern of unknown XX172 and FZD7 with WNT10B-X possibly points to the correlated behaviour with the WNT10B. These higher and lower ranks are guidelines for oncologists/biologists to navigate through the dense and vast combinatorial forest of search space to explore unknown and untested biological hypotheses in the Wnt pathway apropos a subtype of colorectal cancer.

Conflict of interest

There are no conflicts to declare.

Author's contributions

Concept, design, in silico implementation - SS. Analysis and interpretation of results - SS. Manuscript writing - SS. Manuscript revision - SS. Approval of manuscript - SS

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Source of Data

Data used in this research work was released in a publication in [51]. The ETC-1922159 was released in Singapore in July 2015 under the flagship of the Agency for Science, Technology and Research (A*STAR) and Duke-National University of Singapore Graduate Medical School (Duke-NUS).

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Appendix

Choice of sensitivity indices

The SENSITIVITY PACKAGE ([240] and [241]) in R langauge provides a range of functions to compute the indices and the following indices will be taken into account for addressing the posed questions in this manuscript.

1. sensiFdiv - conducts a density-based sensitivity analysis where the impact of an input variable is defined in terms of dissimilarity between the original output density function and the output density function when the input variable is

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fixed. The dissimilarity between density functions is measured with Csiszar f-divergences. Estimation is performed through kernel density estimation and the function kde of the package ks.[242] and[243]

2. sensiHSIC - conducts a sensitivity analysis where the impact of an input variable is defined in terms of the distance between the input/output joint probability distribution and the product of their marginals when they are embedded in a Reproducing Kernel Hilbert Space (RKHS). This distance corresponds to HSIC proposed by[244] and serves as a dependence measure between random variables.

3. soboljansen - implements the Monte Carlo estimation of the Sobol indices for both first-order and total indices at the same time (all together 2p indices), at a total cost of (p+2) × n model evaluations. These are called the Jansen estimators.[245] and[246]

4. sobol2002 - implements the Monte Carlo estimation of the Sobol indices for both first-order and total indices at the same time (all together 2p indices), at a total cost of (p+2) ×n model evaluations. These are called the Saltelli estimators. This estimator suffers from a conditioning problem when estimating the variances behind the indices computations. This can seriously affect the Sobol indices estimates in case of largely non-centered output. To avoid this effect, you have to center the model output before applying "sobol2002". Functions ”soboljansen" and "sobolmartinez" do not suffer from this problem.[247]

5. sobol2007 - implements the Monte Carlo estimation of the Sobol indices for both first-order and total indices at the same time (all together 2p indices), at a total cost of (p+2) × n model evaluations. These are called the Mauntz estimators.[248]

6. sobolmartinez - implements the Monte Carlo estimation of the Sobol indices for both first-order and total indices using correlation coefficients-based formulas, at a total cost of (p + 2) × n model evaluations. These are called the Martinez estimators.

7. sobol - implements the Monte Carlo estimation of the Sobol sensitivity indices. Allows the estimation of the indices of the variance decomposition up to a given order, at a total cost of (N + 1) × n where N is the number of indices to estimate.[249]