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# Machine Learning Discoveries of WNT10B-X Synergy in ETC-1922159 Treated Colorectal Cancer Cells

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# Machine Learning Discoveries of WNT10B-X synergy in ETC-1922159 Treated Colorectal Cancer Cells

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**Abstract:** Wnt family member 10B (WNT10B) is a cysteine-rich glycoprotein, that is a part of the WNT signaling pathway and is implicated in a range of cancers. In colorectal cancer (CRC) cells treated with ETC-1922159, WNT10B was found to be down regulated along with other genes. A recently developed search engine ranked combinations of WNT10B-X (X, a particular gene/protein) at $2^{nd}$ order level after drug administration. Some of these combinations have been tested in wet lab, however many have been pointed out by the search engine that are yet to be explored/tested. These rankings reveal which WNT10B-X combinations might be working synergistically in CRC. In this research work, I cover combinations of WNT10B with mitogen-activated protein kinase (MAPK), GATA zinc finger containing domain antisense RNA (GATA-AS), fatty acid binding protein (FABP), helicase like transcription factor (HLTF), long intergenic non-protein coding RNA (LINC-xxxxx), frizzled class receptor (FZD), homeobox (HOX), cytochrome c oxidase assembly factor (COX), fibroblast growth factor (FGF), interleukin (IL), Rac GTPase (RAC), transforming growth factor beta (TGFB), low density lipoprotein receptor-related protein (LRP), notch receptor (NOTCH), SET and MYND domain containing (SMYD) and forkhead box (FOX) family.

**Keywords:** WNT10B; Porcupine inhibitor ETC-1922159; Sensitivity analysis; Machine learning; Colorectal cancer

## 1. Introduction

### *1.1. 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 Wu et al. [1]. 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 Stevens et al. [2]. Their contribution is also reported in axonal regeneration in injured CNS Tassew et al. [3]. 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 Cawthorn et al. [4] & Collins et al. [5]. 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 Wu et al. [6]. Its implication in breast cancer Wend et al. [7] & Chen et al. [8] as well as endometrial cancer Chen et al. [9] has also been reported.

In colorectal cancer, WNT10B has shown to play a dual function of both oncogenesis promotion via $\beta$-catenin/TCF pathway and the inhibition of cell growth, possibly via FGF family of proteins Yoshikawa et al. [10]. 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 $\beta$-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 Yoshikawa et al. [10]. WNT10B works in tandem with multiple components and some combinations of WNT10B have been

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confirmed in wet lab. However, many of the combinations have not been explored/tested or are known. To reveal these combinations, I use a modification of a recently published machine learning based search engine, details of which are given in the next section.

### *1.2. Combinatorial Search Problem and a Possible Solution*

In a recently published work Sinha [11], a frame work of a search engine was developed which can rank combinations of factors (genes/proteins) in a signaling pathway. Readers are requested to go through the adaptation of the above mentioned work for gaining deeper insight into the working of the pipeline and its use of published data set generated after administration of ETC-1922159, Sinha [12]. The work uses SVM package by Joachims [13] in https://www.cs.cornell.edu/people/tj/svm_light/svm_rank.html. I use the adaptation to rank $2^{nd}$ order gene combinations.

## 2. Results & Discussion

### *2.1. WNT10B Related Synergies*

#### 2.1.1. WNT10B - MAPK15 / GATA6-AS1 / FABP5 / HLTF

Chen et al. [14] demonstrate that WNT10B provides defense mechanisms against doxorubicin-induced cardiotoxicity and apoptosis, via MAPK signaling. Khalid et al. [15] show that in GATA4 knockout mice bone marrow derived mesenchymal stem cells had reduction in WNT ligands, particularly WNT10B as compared to control cells. They demonstrated that GATA4 is recruited to enhancers near WNT10B, thus regulating the WNT signalosome. In glioblastoma cells, Li et al. [16] exhibited a high level of FABP4 expression, and down-regulation of FABP4 suppressed tumor cell growth and metastasis. Further it was found that WNT10b, a regulator gene of FABP4, restored the effects of FABP4 down-regulation in glioblastoma cells. Helmer et al. [17] observe that HLTF regulates WNT10B signaling in brain. All these experimental validations point to existing combinatorial synergy between the involved members and WNT10B. In colorectal cancer cells treated with ETC-1922159, individual family members and WNT10B, were found to be down regulated and their regulation was recorded independently. I was able to rank $2^{nd}$ order combination of these individual members along with WBT10B.

Table 1 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in Table 2 generated from analysis of the ranks in Table 1. The Table 1 shows rankings of individual members w.r.t WNT10B. HLTF - WNT10B shows low ranking of 1162 (linear) and 1137 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment. Interestingly, MAPK15, GATA6-AS1 and FABP5 showed high ranking with WNT10B, thus indicating that they might not be working synergistically with WNT10B, before the drug treatment.

**Table 1.** $2^{nd}$ order interaction ranking between WNT10B VS individual members.

| RANKING INDIVIDUAL MEMBERS VS WNT10B | | | |
|---|---|---|---|
| RANKING OF INDIVIDUAL MEMEBRS W.R.T WNT10B | | | |
| | laplace | linear | rbf |
| MAPK15 - WNT10B | 574 | 2470 | 2147 |
| GATA6-AS1 - WNT10B | 475 | 2031 | 2240 |
| FABP5 - WNT10B | 277 | 2222 | 2461 |
| HLTF - WNT10B | 1816 | 1162 | 1137 |

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One can also interpret the results of the Table 1 graphically, with the following influences - • individual members w.r.t WNT10B with WNT10B $->$ HLTF.

**Table 2.** $2^{nd}$ order combinatorial hypotheses between WNT10B and individual members.

| UNEXPLORED COMBINATORIAL HYPOTHESES | |
|---|---|
| Individual members w.r.t WNT10B | |
| HLTF | WNT10B |

2.1.2. WNT10B - LINC-xxxxx

In colorectal cancer cells, Xi et al. [18] demostrated that inhibition of LINC00261 caused an increase in microRNA-148a expression and a decrease in expression of WNT10B and $\beta$-catenin. These indicate that LINC00261 may affect colon cancer progression by modulating the miR-148a/WNT10b axis. In colorectal cancer cells treated with ETC-1922159, LINC-xxxxx family members and WNT10B, were found to be down regulated and their regulation was recorded independently. I was able to rank $2^{nd}$ order combination of these LINC-xxxxx members along with WBT10B.

Table 3 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in Table 4 generated from analysis of the ranks in Table 3. The Table 3 shows rankings of LINC-xxxxx members w.r.t WNT10B. LINC00261 - WNT10B shows low ranking of 651 (laplace) and 1513 (rbf). LINC01123 - WNT10B shows low ranking of 1126 (laplace) and 1528 (rbf). LINC00888 - WNT10B shows low ranking of 1216 (laplace), 961 (linear) and 898 (rbf). LINC00338 - WNT10B shows low ranking of 530 (linear) and 543 (rbf). LINC00242 - WNT10B shows low ranking of 754 (linear) and 1001 (rbf). LINC00858 - WNT10B shows low ranking of 860 (linear) and 769 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, LINC01106 showed high ranking with WNT10B, thus indicating that they might not be working synergistically with WNT10B, before the drug treatment.

**Table 3.** $2^{nd}$ order interaction ranking between WNT10B VS LINC-xxxxx members.

| RANKING LINC-XXXXX FAMILY VS WNT10B | | | | | | | |
|---|---|---|---|---|---|---|---|
| | RANKING OF LINC-XXXXX FAMILY W.R.T WNT10B | | | | | | |
| | laplace | linear | rbf | | laplace | linear | rbf |
| LINC01106 - WNT10B | 503 | 2295 | 1998 | LINC00261 - WNT10B | 651 | 1767 | 1513 |
| LINC01123 - WNT10B | 1126 | 1787 | 1528 | LINC00888 - WNT10B | 1216 | 961 | 898 |
| LINC00338 - WNT10B | 1651 | 530 | 543 | LINC00242 - WNT10B | 2258 | 754 | 1001 |
| LINC00858 - WNT10B | 2364 | 860 | 769 | | | | |

One can also interpret the results of the Table 3 graphically, with the following influences - • LINC-xxxxx members w.r.t WNT10B with WNT10B $->$ LINC-00261 / 01123 / 00888 / 00338 / 00242 / 00858.

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**Table 4.** $2^{nd}$ order combinatorial hypotheses between WNT10B and LINC-xxxxx members.

| UNEXPLORED COMBINATORIAL HYPOTHESES |
|---|
| LINC-xxxxx members w.r.t WNT10B |
| LINC-00261/01123/00888/00338/00242/00858 - WNT10B |

2.1.3. WNT10B - FZD

In T-cell acute lymphoblastic leukemia, using MOLT4 and MUTZ-2 as leukemic cell models characterized by the expression of WNT10B$^{IVS1}$, Cassaro et al. [19] observed that WNT10B drives WNT activation via FZD6 receptor ligand binding. In colorectal cancer cells treated with ETC-1922159, FZD family members and WNT10B, were found to be down regulated and their regulation was recorded independently. I was able to rank $2^{nd}$ order combination of these FZD members along with WBT10B.

Table 5 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in Table 6 generated from analysis of the ranks in Table 5. The Table 5 shows rankings of FZD members w.r.t WNT10B. FZD7 - WNT10B shows low ranking of 1121 (laplace), 1331 (linear) and 1395 (rbf). FZD3 - WNT10B shows low ranking of 724 (linear) and 727 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

**Table 5.** $2^{nd}$ order interaction ranking between WNT10B VS FZD members.

| RANKING FZD FAMILY VS WNT10B | | | |
|---|---|---|---|
| RANKING OF FZD FAMILY W.R.T WNT10B | | | |
| | laplace | linear | rbf |
| FZD7 - WNT10B | 1121 | 1331 | 1395 |
| FZD3 - WNT10B | 1805 | 724 | 727 |

One can also interpret the results of the Table 5 graphically, with the following influences - • FZD members w.r.t WNT10B with WNT10B $->$ FZD-7/3.

**Table 6.** $2^{nd}$ order combinatorial hypotheses between WNT10B and FZD members.

| UNEXPLORED COMBINATORIAL HYPOTHESES |
|---|
| FZD members w.r.t WNT10B |
| FZD-7/3 - WNT10B |

2.1.4. WNT10B - HOX

In oral squamous cell carcinoma cells, Dai et al. [20] found an enhanced migration ability induced by WNT10B, but it was reversed by HOXC10 knockdown. In colorectal cancer cells treated with ETC-1922159, HOX family members and WNT10B, were found to be down regulated and their regulation

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was recorded independently. I was able to rank $2^{nd}$ order combination of these HOX members along with WBT10B.

Table 7 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in Table 8 generated from analysis of the ranks in Table 7. The Table 7 shows rankings of HOX members w.r.t WNT10B. HOXB8 - WNT10B shows low ranking of 1233 (laplace), 1144 (linear) and 1487 (rbf). HOXB9 - WNT10B shows low ranking of 1413 (laplace), 1264 (linear) and 1321 (rbf). HOXB4 - WNT10B shows low ranking of 1554 (laplace), 1579 (linear) and 1354 (rbf). HOXA9 - WNT10B shows low ranking of 1556 (laplace), 518 (linear) and 668 (rbf). HOXB5 - WNT10B shows low ranking of 296 (linear) and 481 (rbf). HOXA11 - WNT10B shows low ranking of 74 (linear) and 584 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, HOXB13, HOXB7, HOXB3 and HOXA11-AS showed high ranking with WNT10B, thus indicating that they might not be working synergistically with WNT10B, before the drug treatment.

**Table 7.** $2^{nd}$ order interaction ranking between WNT10B VS HOX members.

| Ranking HOX family VS WNT10B | | | | | | | |
|---|---|---|---|---|---|---|---|
| Ranking of HOX family w.r.t WNT10B | | | | | | | |
| | laplace | linear | rbf | | laplace | linear | rbf |
| HOXB13 - WNT10B | 82 | 2417 | 2583 | HOXB7 - WNT10B | 84 | 2709 | 2705 |
| HOXB3 - WNT10B | 838 | 1950 | 1912 | HOXA11-AS - WNT10B | 860 | 2021 | 1866 |
| HOXB8 - WNT10B | 1233 | 1144 | 1487 | HOXB9 - WNT10B | 1413 | 1264 | 1321 |
| HOXB4 - WNT10B | 1554 | 1579 | 1354 | HOXA9 - WNT10B | 1556 | 518 | 668 |
| HOXB5 - WNT10B | 1950 | 296 | 481 | HOXA11 - WNT10B | 2612 | 74 | 584 |

One can also interpret the results of the Table 7 graphically, with the following influences - • HOX members w.r.t WNT10B with WNT10B $->$ HOX-B8/B9/B4/A9/B5/A11.

**Table 8.** $2^{nd}$ order combinatorial hypotheses between WNT10B and HOX members.

| Unexplored combinatorial hypotheses | |
|---|---|
| HOX members w.r.t WNT10B | |
| HOX-B8/B9/B4/A9/B5/A11 | WNT10B |

2.1.5. WNT10B - COX

WNT10B can promote bone morphogenetic protein 9 (BMP9) induced osteogenic differentiation via the COX2/p-CREB dependent manner, as obaerved by Liao et al. [21]. In colorectal cancer cells treated with ETC-1922159, COX family members and WNT10B, were found to be down regulated and their regulation was recorded independently. I was able to rank $2^{nd}$ order combination of these COX members along with WBT10B.

Table 9 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in Table 10 generated from analysis of the ranks in Table 9. The Table 9 shows rankings of COX members w.r.t WNT10B. COX10-AS1 - WNT10B shows low ranking of 1429 (linear) and 1261 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, COX10, COX14 and COX18 showed high ranking with WNT10B, thus indicating that they might not be working synergistically with WNT10B, before the drug treatment.

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**Table 9.** $2^{nd}$ order interaction ranking between WNT10B VS COX members.

| RANKING COX FAMILY VS WNT10B | | | |
|---|---|---|---|
| RANKING OF COX FAMILY W.R.T WNT10B | | | |
| | laplace | linear | rbf |
| COX10 - WNT10B | 13 | 2300 | 2219 |
| COX14 - WNT10B | 448 | 2178 | 2186 |
| COX18 - WNT10B | 611 | 2224 | 2135 |
| COX10-AS1 - WNT10B | 1757 | 1429 | 1261 |

One can also interpret the results of the Table 9 graphically, with the following influences - • COX members w.r.t WNT10B with WNT10B $-$ > COX10-AS1.

**Table 10.** $2^{nd}$ order combinatorial hypotheses between WNT10B and COX members.

| UNEXPLORED COMBINATORIAL HYPOTHESES | |
|---|---|
| COX members w.r.t WNT10B | |
| COX-10-AS1 | WNT10B |

2.1.6. WNT10B - FGF

Xiao et al. [22] support the hypothesis that the impaired anabolic response to parathyroid hormone in the absence of endogenous FGF2 is due in part to attenuated WNT signaling. WNT10B expression was altered by FGF2 deficiency. In colorectal cancer cells treated with ETC-1922159, FGF family members and WNT10B, were found to be down regulated and their regulation was recorded independently. I was able to rank $2^{nd}$ order combination of these FGF members along with WBT10B.

Table 11 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in Table 12 generated from analysis of the ranks in Table 11. The Table 11 shows rankings of FGF members w.r.t WNT10B. FGF2 - WNT10B shows low ranking of 1020 (laplace), 1223 (linear) and 1155 (rbf). FGFBP3 - WNT10B shows low ranking of 740 (linear) and 384 (rbf). FGFR4 - WNT10B shows low ranking of 454 (linear) and 716 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

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**Table 11.** $2^{nd}$ order interaction ranking between WNT10B VS FGF members.

| RANKING FGF FAMILY VS WNT10B | | | |
|---|---|---|---|
| RANKING OF FGF FAMILY W.R.T WNT10B | | | |
| | laplace | linear | rbf |
| FGF2 - WNT10B | 1020 | 1223 | 1155 |
| FGFBP3 - WNT10B | 2353 | 740 | 384 |
| FGFR4 - WNT10B | 2403 | 454 | 716 |

One can also interpret the results of the Table 11 graphically, with the following influences - • FGF members w.r.t WNT10B with WNT10B $-$ > FGF-2/BP3/R4.

**Table 12.** $2^{nd}$ order combinatorial hypotheses between WNT10B and FGF members.

| UNEXPLORED COMBINATORIAL HYPOTHESES | |
|---|---|
| FGF members w.r.t WNT10B | |
| FGF-2/BP3/R4 | WNT10B |

2.1.7. WNT10B - IL

He et al. [23] observed high level of IL17A promoted mesenchymal stem cells-2 polarization through WNT10B/RUNX2 pathway in new bone formation developed in ankylosing spondylitis. In colorectal cancer cells treated with ETC-1922159, IL family members and WNT10B, were found to be down regulated and their regulation was recorded independently. I was able to rank $2^{nd}$ order combination of these IL members along with WBT10B.

Table 13 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in Table 14 generated from analysis of the ranks in Table 13. The Table 13 shows rankings of IL members w.r.t WNT10B. IL1RL2 - WNT10B shows low ranking of 991 (laplace) and 1502 (rbf). IL17D - WNT10B shows low ranking of 1546 (laplace), 762 (linear) and 706 (rbf). IL17RB - WNT10B shows low ranking of 825 (linear) and 573 (rbf). ILF3 - WNT10B shows low ranking of 343 (linear) and 412 (rbf). ILF3-AS1 - WNT10B shows low ranking of 591 (linear) and 631 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, IL17RD, ILF2 and IL33 showed high ranking with WNT10B, thus indicating that they might not be working synergistically with WNT10B, before the drug treatment.

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**Table 13.** $2^{nd}$ order interaction ranking between WNT10B VS IL members.

| RANKING IL FAMILY VS WNT10B | | | |
|---|---|---|---|
| RANKING OF IL FAMILY W.R.T WNT10B | | | |
| | laplace | linear | rbf |
| IL17RD - WNT10B | 330 | 2190 | 2407 |
| ILF2 - WNT10B | 529 | 2509 | 2338 |
| IL1RL2 - WNT10B | 991 | 1871 | 1502 |
| IL33 - WNT10B | 1118 | 1562 | 1694 |
| IL17D - WNT10B | 1546 | 762 | 706 |
| IL17RB - WNT10B | 1829 | 825 | 573 |
| ILF3 - WNT10B | 2231 | 343 | 412 |
| ILF3-AS1 - WNT10B | 2629 | 591 | 631 |

One can also interpret the results of the Table 13 graphically, with the following influences - • IL members w.r.t WNT10B with WNT10B $->$ IL-1RL2/17D/17RB/F3/F3-AS1.

**Table 14.** $2^{nd}$ order combinatorial hypotheses between WNT10B and IL members.

| UNEXPLORED COMBINATORIAL HYPOTHESES | |
|---|---|
| IL members w.r.t WNT10B | |
| IL-1RL2/17D/17RB/F3/F3-AS1 | WNT10B |

2.1.8. WNT10B - RAC

Lee and Heur [24] observe that as WNT ligands regulate many critical cellular functions, such as proliferation, they might be attractive candidates for modulation in human corneal endothelial dysfunction. The author's show that WNT10B causes nuclear transport and binding of RAC1 and $\beta$-catenin in human corneal endothelial cells, leading to the activation of cyclin D1 expression and proliferation. In colorectal cancer cells treated with ETC-1922159, RAC family members and WNT10B, were found to be down regulated and their regulation was recorded independently. I was able to rank $2^{nd}$ order combination of these RAC members along with WBT10B.

Table 15 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in Table 16 generated from analysis of the ranks in Table 15. The Table 15 shows rankings of RAC members w.r.t WNT10B. RAC3 - WNT10B shows low ranking of 1419 (linear) and 1349 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

 doi:10.20944/preprints202501.0674.v1



Further, RACGAP1 showed high ranking with WNT10B, thus indicating that they might not be working synergistically with WNT10B, before the drug treatment.

**Table 15.** $2^{nd}$ order interaction ranking between WNT10B VS RAC members.

| Ranking RAC family VS WNT10B | | | |
|---|---|---|---|
| Ranking of RAC family w.r.t WNT10B | | | |
| | laplace | linear | rbf |
| RAC3 - WNT10B | 1682 | 1419 | 1349 |
| RACGAP1 - WNT10B | 586 | 2072 | 1851 |

One can also interpret the results of the Table 15 graphically, with the following influences - • RAC members w.r.t WNT10B with WNT10B $-$ > RAC-3.

**Table 16.** $2^{nd}$ order combinatorial hypotheses between WNT10B and RAC members.

| Unexplored combinatorial hypotheses | |
|---|---|
| RAC members w.r.t WNT10B | |
| RAC-3 | WNT10B |

2.1.9. WNT10B - TGFB

Ota et al. [25] have documented that osteoclasts secrete a homolog of Drosophila WNT10B, bone morphogenetic protein 6 (BMP6), and the chemokine sphingosin 1 phosphate (S1P) to promote mesenchymal cell mineralization in vitro. During bone resorption, TGF$\beta$1 is released from the bone extracellular matrix and activated by osteoclasts which increased osteoclast production of WNT10B, but not BMP6 or S1P. These results demonstrated that TGF$\beta$1 stimulates WNT10B production in osteoclasts thus augment restoration of the bone lost during the resorptive phase of bone turnover. In colorectal cancer cells treated with ETC-1922159, TGFB family members and WNT10B, were found to be down regulated and their regulation was recorded independently. I was able to rank $2^{nd}$ order combination of these TGFB members along with WBT10B.

Table 17 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in Table 18 generated from analysis of the ranks in Table 17. The Table 17 shows rankings of TGFB members w.r.t WNT10B. TGFBR3 - WNT10B shows low ranking of 1539 (laplace), 1228 (linear) and 1270 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, TGFBRAP1 and TGFB1 showed high ranking with WNT10B, thus indicating that they might not be working synergistically with WNT10B, before the drug treatment.

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**Table 17.** $2^{nd}$ order interaction ranking between WNT10B VS TGFB members.

| RANKING TGFB FAMILY VS WNT10B | | | |
|---|---|---|---|
| RANKING OF TGFB FAMILY W.R.T WNT10B | | | |
| | laplace | linear | rbf |
| TGFBRAP1 - WNT10B | 682 | 2077 | 2106 |
| TGFB1 - WNT10B | 1514 | 1978 | 1905 |
| TGFBR3 - WNT10B | 1539 | 1228 | 1270 |

One can also interpret the results of the Table 17 graphically, with the following influences - • TGFB members w.r.t WNT10B with WNT10B $-$ > TGFB-R3.

**Table 18.** $2^{nd}$ order combinatorial hypotheses between WNT10B and TGFB members.

| UNEXPLORED COMBINATORIAL HYPOTHESES | |
|---|---|
| TGFB members w.r.t WNT10B | |
| TGFB-R3 | WNT10B |

2.1.10. WNT10B - LRP

Goel et al. [26] show that adult mammary ductal stem cell activity is maintained by canonical WNT signal which requires both LRP5 and LRP6. The canonical ligands WNT9B and WNT10B are expressed in mammary gland. In colorectal cancer cells treated with ETC-1922159, LRP family members and WNT10B, were found to be down regulated and their regulation was recorded independently. I was able to rank $2^{nd}$ order combination of these LRP members along with WBT10B.

Table 19 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in Table 20 generated from analysis of the ranks in Table 19. The Table 19 shows rankings of LRP members w.r.t WNT10B. LRP3 - WNT10B shows low ranking of 895 (linear) and 1141 (rbf). LRP8 - WNT10B shows low ranking of 515 (linear) and 362 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

**Table 19.** $2^{nd}$ order interaction ranking between WNT10B VS LRP members.

| RANKING LRP FAMILY VS WNT10B | | | |
|---|---|---|---|
| RANKING OF LRP FAMILY W.R.T WNT10B | | | |
| | laplace | linear | rbf |
| LRP3 - WNT10B | 1680 | 895 | 1141 |
| LRP8 - WNT10B | 2252 | 515 | 362 |

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One can also interpret the results of the table 19 graphically, with the following influences - • LRP members w.r.t WNT10B with WNT10B $->$ LRP-3/8.

**Table 20.** $2^{nd}$ order combinatorial hypotheses between WNT10B and LRP members.

| UNEXPLORED COMBINATORIAL HYPOTHESES | |
|---|---|
| LRP members w.r.t WNT10B | |
| LRP-3/8 | WNT10B |

2.1.11. WNT10B - NOTCH

In human U2OS osteosarcoma cells, Mödder et al. [27] demostrated that WNT10B stimulated the NF$\kappa$B and NOTCH pathways, which play important role in bone biology. In colorectal cancer cells treated with ETC-1922159, NOTCH family members and WNT10B, were found to be down regulated and their regulation was recorded independently. I was able to rank $2^{nd}$ order combination of these NOTCH members along with WBT10B.

Table 21 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in Table 22 generated from analysis of the ranks in Table 21. The Table 21 shows rankings of NOTCH members w.r.t WNT10B. NOTCH4 - WNT10B shows low ranking of 1223 (laplace) and 1104 (rbf). NOTCH1 - WNT10B shows low ranking of 308 (linear) and 388 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

**Table 21.** $2^{nd}$ order interaction ranking between WNT10B VS NOTCH members.

| RANKING NOTCH FAMILY VS WNT10B | | | |
|---|---|---|---|
| RANKING OF NOTCH FAMILY W.R.T WNT10B | | | |
| | laplace | linear | rbf |
| NOTCH4 - WNT10B | 1223 | 1631 | 1104 |
| NOTCH1 - WNT10B | 1711 | 308 | 388 |

One can also interpret the results of the Table 21 graphically, with the following influences - • NOTCH members w.r.t WNT10B with WNT10B $->$ NOTCH-4/1.

**Table 22.** $2^{nd}$ order combinatorial hypotheses between WNT10B and NOTCH members.

| UNEXPLORED COMBINATORIAL HYPOTHESES | |
|---|---|
| NOTCH members w.r.t WNT10B | |
| NOTCH-4/1 | WNT10B |

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2.1.12. WNT10B - SMYD

Luo et al. [28] showed that SMYD3 was found to encode a novel histone methyltransferase involved in human cancer cells and activate the transcription of oncogene WNT10B, in mouse embryo fibroblast NIH3T3 cell line. In colorectal cancer cells treated with ETC-1922159, SMYD family members and WNT10B, were found to be down regulated and their regulation was recorded independently. I was able to rank $2^{nd}$ order combination of these SMYD members along with WBT10B.

Table 23 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in Table 24 generated from analysis of the ranks in Table 23. The Table 23 shows rankings of SMYD members w.r.t WNT10B. SMYD5 - WNT10B shows low ranking of 985 (linear) and 1209 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, SMYD3 showed high ranking with WNT10B, thus indicating that they might not be working synergistically with WNT10B, before the drug treatment.

**Table 23.** $2^{nd}$ order interaction ranking between WNT10B VS SMYD members.

| RANKING SMYD FAMILY VS WNT10B | | | |
|---|---|---|---|
| RANKING OF SMYD FAMILY W.R.T WNT10B | | | |
| | laplace | linear | rbf |
| SMYD3 - WNT10B | 1464 | 1859 | 1714 |
| SMYD5 - WNT10B | 1606 | 985 | 1209 |

One can also interpret the results of the Table 23 graphically, with the following influences - • SMYD members w.r.t WNT10B with WNT10B $->$ SMYD-5.

**Table 24.** $2^{nd}$ order combinatorial hypotheses between WNT10B and SMYD members.

| UNEXPLORED COMBINATORIAL HYPOTHESES | |
|---|---|
| SMYD members w.r.t WNT10B | |
| SMYD-5 | WNT10B |

2.1.13. WNT10B - FOX

Gerin et al. [29] show that WNT10B is induced by FOX transcription factors but it appears that increased WNT signaling alone is not sufficient to explain the mechanism whereby FOX transcription factors repress adipogenesis. In colorectal cancer cells treated with ETC-1922159, FOX family members and WNT10B, were found to be down regulated and their regulation was recorded independently. I was able to rank $2^{nd}$ order combination of these FOX members along with WBT10B.

Table 25 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in Table 26 generated from analysis of the ranks in Table 25. The Table 25 shows rankings of FOX members w.r.t WNT10B. FOXM1 - WNT10B shows low ranking of 619 (laplace), 1494 (linear) and 1371 (rbf). FOXJ1 - WNT10B shows low ranking of 516 (linear) and 467 (rbf). FOXA2 - WNT10B shows low ranking of 30 (linear) and 43 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

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Further, FOXD2-AS1 showed high ranking with WNT10B, thus indicating that they might not be working synergistically with WNT10B, before the drug treatment.

**Table 25.** $2^{nd}$ order interaction ranking between WNT10B VS FOX members.

| RANKING FOX FAMILY VS WNT10B | | | |
|---|---|---|---|
| RANKING OF FOX FAMILY W.R.T WNT10B | | | |
| | laplace | linear | rbf |
| FOXD2-AS1 - WNT10B | 331 | 2246 | 2006 |
| FOXM1 - WNT10B | 619 | 1494 | 1371 |
| FOXJ1 - WNT10B | 1782 | 516 | 467 |
| FOXA2 - WNT10B | 2573 | 30 | 43 |

One can also interpret the results of the Table 25 graphically, with the following influences - • FOX members w.r.t WNT10B with WNT10B $->$ FOX-M1/J1/A2.

**Table 26.** $2^{nd}$ order combinatorial hypotheses between WNT10B and FOX members.

| UNEXPLORED COMBINATORIAL HYPOTHESES | |
|---|---|
| FOX members w.r.t WNT10B | |
| FOX-M1/J1/A2 | WNT10B |

## 3. Conclusions

Presented here are a range of multiple synergistic WNT10B $2^{nd}$ order combinations that were ranked via a machine learning based search engine. Via majority voting across the ranking methods, it was possible to find plausible unexplored synergistic combinations of WNT10B-X that might be prevalent in CRC cells after treatment with ETC-1922159 drug.

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## Source of Data

Data used in this research work was released in a publication in Madan et al. [30].

**Conflicts of Interest:** There are no conflicts to declare.

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