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

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Article

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

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Abstract: Wntless (WLS) is a receptor required for WNT secretion. WLS is a cargo for the retromer complex. In the absence of retromer, WLS is degraded in lysosomes and the WNT secretion is impaired. In colorectal cancer (CRC) cells treated with ETC-1922159, WLS was found to be up regulated along with other genes. A recently developed search engine ranked combinations of WLS-X (X, a particular gene/protein) at 2^{nd} order level after drug administration. Some combinations have been experimentally validated, while many remain untested/unexplored. These rankings reveal which WLS-X combinations might be working synergistically in CRC. In this research work, I cover combinations of WLS with WNT, prolactin regulatory element-binding protein (SEC12), member RAS oncogene family (RAB), vacuolar protein sorting protein, a component of the retromer complex (VPS), sorting nexin (SNX), ADP ribosylation factors (ARF), ubiquitin conjugating enzyme E2 (UBE2), ATPases and transmembrane protein (TMEM) family.

Keywords: WLS; porcupine inhibitor ETC-1922159; sensitivity analysis; colorectal cancer

1. Introduction

1.1. Wnt Secretion

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 Tanaka et al. [1]. It is believed that these ligands are then transported via the EVI/WNTLESS transmembrane complex out of the cell (Banziger et al. [2], Bartscherer et al. [3] and Goodman et al. [4]). The EVI/WNTLESS themselves are known to reside in the Golgi bodies and interaction with the WNT ligands for the later's glycosylation Kurayoshi et al. [5] & Gao and Hannoush [6]. 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.

Voloshanenko et al. [7] show that WLS and WNT3 are highly expressed in colon carcinomas and EVI/WLS is required for high levels of WNT pathway activation. Further, Chua et al. [8] demonstrate that clinical osteosarcoma samples show high WLS and β-catenin expression. In colorectal cancer cells treated with ETC-1922159, WLS was found to be up regulated along with other genes. Some of the WLS-X (X, a particular gene/protein) combinations have been experimentally validated, while many remain untested/unexplored. I use the machine learning based search engine (the next section) to rank/prioritize these combinations to reveal untested/unexplored combinations.

1.2. Combinatorial Search Problem and a Possible Solution

In a recently published work Sinha [9], 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

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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 [10]. The work uses SVM package by Joachims [11] 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. WLS Related Synergies

2.1.1. WLS-SEC

Sun et al. [12] show that WLS forms a complex with SEC12. Binding of mature WNT to WLS increases WLS-SEC12 interaction and promotes association of WLS with SAR1 (a key activator of the COPII machinery). Mutant WLS that fail to communicate with the COPII machinery cannot effectively support WNT secretion. In colorectal cancer cells treated with ETC-1922159, SEC family and WLS, were found to be up regulated and recorded independently. I was able to rank 2^{nd} order combination of SEC family and WLS, that were up regulated.

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 SEC family w.r.t WLS. SEC24C - WLS shows low ranking of 1560 (laplace) and 622 (rbf). This low ranking points to the fact that the combination is not relevant after ETC-1922159 treatment of CRC, however, it might be prevalent in CRC, before treatment.

Further, SEC24D - WLS show high ranking of 1644 (laplace), 2092 (linear) and 1679 (rbf). SEC31A - WLS show high ranking of 1856 (laplace) and 1793 (linear). This high ranking points to the fact that the combination is relevant after ETC-1922159 treatment of CRC, however, it might not be prevalent in CRC, before treatment.

Table 1. 2^{nd} order interaction ranking between WLS VS SEC family members.

RANKING SEC FAMILY VS WLS			
RANKING OF SEC FAMILY W.R.T WLS			
	laplace	linear	rbf
SEC24C - WLS	1560	2089	622
SEC24D - WLS	1644	2092	1679
SEC31A - WLS	1856	1793	831

One can also interpret the results of the table 1 graphically, with the following influences - • SEC family w.r.t WLS with WLS $->$ SEC-24C/24D/31A.

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Table 2. 2^{nd} order combinatorial hypotheses between WLS and SEC family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
SEC family w.r.t WLS	
SEC-24C	WLS (before ETC-1922159 treatment of CRC)
SEC-24D/31A	WLS (after ETC-1922159 treatment of CRC)

2.1.2. WLS-RAB

Das et al. [13] show that WNT secretion is dependent on RAB8A mediated transport of GPR177 (WNTLESS). GPR177 was found to bind with RAB8A, depletion of which compromised GPR177 traffic, thereby weakening the secretion of multiple WNTs. Further, Sun et al. [12] tabulate proteomic identification of RAB family in wild-type WLS and WLS^{1-491} immunoprecipitates. In colorectal cancer cells treated with ETC-1922159, RAB family and WLS, were found to be up regulated and recorded independently. I was able to rank 2^{nd} order combination of RAB family and WLS, that were up regulated.

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 RAB family w.r.t WLS. RAB24 - WLS shows low ranking of 249 (laplace), 739 (linear) and 1344 (rbf). RAB3B - WLS shows low ranking of 642 (laplace), 459 (linear) and 828 (rbf). RAB22A - WLS shows low ranking of 757 (laplace), 657 (linear) and 1078 (rbf). RAB5A - WLS shows low ranking of 771 (laplace) and 267 (rbf). RAB9A - WLS shows low ranking of 858 (laplace), 1562 (linear) and 371 (rbf). RAB4B - WLS shows low ranking of 1273 (laplace) and 572 (linear). RAB8A - WLS shows low ranking of 919 (linear) and 917 (rbf). RAB1A - WLS shows low ranking of 658 (linear) and 1535 (rbf). This low ranking points to the fact that the combination is not relevant after ETC-1922159 treatment of CRC, however, it might be prevalent in CRC, before treatment.

Further, RAB25 - WLS show high ranking of 1939 (linear) and 2498 (rbf). RAB3GAP1 - WLS show high ranking of 2438 (linear) and 2103 (rbf). RAB11FIP1 - WLS show high ranking of 1561 (laplace) and 1653 (linear). RAB7A - WLS show high ranking of 1870 (laplace) and 2465 (rbf). RAB11A - WLS show high ranking of 2135 (laplace) and 2148 (linear). RAB1B - WLS show high ranking of 2237 (laplace), 2002 (linear) and 1730 (rbf). This high ranking points to the fact that the combination is relevant after ETC-1922159 treatment of CRC, however, it might not be prevalent in CRC, before treatment.

Table 3. 2^{nd} order interaction ranking between WLS VS RAB family members.

RANKING RAB FAMILY VS WLS							
	RANKING OF RAB FAMILY W.R.T WLS						
	laplace	linear	rbf		laplace	linear	rbf
RAB24 - WLS	249	739	1344	RAB3B - WLS	642	459	828
RAB22A - WLS	757	657	1078	RAB5A - WLS	771	1688	267
RAB9A - WLS	858	1562	371	RAB4B - WLS	1273	572	2021
RAB25 - WLS	1445	1939	2498	RAB3GAP1 - WLS	1452	2438	2103
RAB11FIP1 - WLS	1561	1653	185	RAB8A - WLS	1751	919	917
RAB7A - WLS	1870	959	2465	RAB11A - WLS	2135	2148	1177
RAB1B - WLS	2237	2002	1730	RAB1A - WLS	2394	658	1535

One can also interpret the results of the table 3 graphically, with the following influences - • RAB family w.r.t WLS with $WLS -> RAB\text{-}24/3B/22A/5A/9A/4B/8A/1A$ (before ETC-1922159 treatment of CRC) and $WLS -> RAB\text{-}25/3GAP1/11FIP1/7A/11A/1B$ (after ETC-1922159 treatment of CRC).

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Table 4. 2^{nd} order combinatorial hypotheses between WLS and RAB family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
RAB family w.r.t WLS	
RAB-24/3B/22A/5A/9A/4B/8A/1A	WLS (before ETC-1922159 treatment of CRC)
RAB-25/3GAP1/11FIP1/7A/11A/1B	WLS (after ETC-1922159 treatment of CRC)

2.1.3. WLS-VPS

Belenkaya et al. [14] examined the role of VPS35 (a retromer subunit), in WNT signaling. They provide compelling evidence that VPS35 colocalizes in endosomes and interacts with WLS and WLS becomes unstable in the absence of retromer activity. Further, Sun et al. [12] tabulate proteomic identification of VPS family in wild-type WLS and WLS^{1-491} immunoprecipitates. In colorectal cancer cells treated with ETC-1922159, VPS family and WLS, were found to be up regulated and recorded independently. I was able to rank 2^{nd} order combination of VPS family and WLS, that were up regulated.

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 VPS family w.r.t WLS. VPS37C - WLS shows low ranking of 1305 (laplace), 1381 (linear) and 1208 (rbf). VPS33B - WLS shows low ranking of 967 (linear) and 604 (rbf). This low ranking points to the fact that the combination is not relevant after ETC-1922159 treatment of CRC, however, it might be prevalent in CRC, before treatment.

Further, VPS37B - WLS show high ranking of 1715 (laplace), 2182 (linear) and 1821 (rbf). VPS28 - WLS show high ranking of 2091 (laplace) and 2453 (rbf). VPS4B - WLS show high ranking of 2487 (laplace) and 1872 (rbf). This high ranking points to the fact that the combination is relevant after ETC-1922159 treatment of CRC, however, it might not be prevalent in CRC, before treatment.

Table 5. 2^{nd} order interaction ranking between WLS VS VPS family members.

RANKING VPS FAMILY VS WLS			
RANKING OF VPS FAMILY W.R.T WLS			
	laplace	linear	rbf
VPS37C - WLS	1305	1381	1208
VPS37B - WLS	1715	2182	1821
VPS33B - WLS	1777	967	604
VPS28 - WLS	2091	599	2453
VPS4B - WLS	2487	1028	1872

One can also interpret the results of the table 5 graphically, with the following influences - • VPS family w.r.t WLS with $WLS -> VPS\text{-}37C/33B$ (before ETC-1922159 treatment of CRC) and $WLS -> VPS\text{-}37B/28/4B$ (after ETC-1922159 treatment of CRC).

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Table 6. 2^{nd} order combinatorial hypotheses between WLS and VPS family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
VPS family w.r.t WLS	
VPS-37C/33B	WLS (before ETC-1922159 treatment of CRC)
VPS-37B/28/4B	WLS (after ETC-1922159 treatment of CRC)

2.1.4. WLS-SNX

Harterink et al. [15] found that SNX3 has an evolutionarily conserved function in WLS recycling and WNT secretion. Brown et al. [16] show similar findings about SNX3 and WLS in mammalian neural tube closure. In colorectal cancer cells treated with ETC-1922159, SNX family and WLS, were found to be up regulated and recorded independently. I was able to rank 2^{nd} order combination of SNX family and WLS, that were up regulated.

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 SNX family w.r.t WLS. SNX9 - WLS shows low ranking of 229 (laplace), 19 (linear) and 1501 (rbf). SNX11 - WLS shows low ranking of 1159 (laplace) and 1353 (rbf). This low ranking points to the fact that the combination is not relevant after ETC-1922159 treatment of CRC, however, it might be prevalent in CRC, before treatment.

Further, SNX33 - WLS show high ranking of 1948 (laplace) and 2378 (linear). This high ranking points to the fact that the combination is relevant after ETC-1922159 treatment of CRC, however, it might not be prevalent in CRC, before treatment.

Table 7. 2^{nd} order interaction ranking between WLS VS SNX family members.

RANKING SNX FAMILY VS WLS			
RANKING OF SNX FAMILY W.R.T WLS			
	laplace	linear	rbf
SNX9 - WLS	229	19	1501
SNX11 - WLS	1159	1691	1353
SNX33 - WLS	1948	2378	53

One can also interpret the results of the table 7 graphically, with the following influences - • SNX family w.r.t WLS with $WLS -> SNX\text{-}9/11$ (before ETC-1922159 treatment of CRC) and $WLS -> SNX\text{-}33$ (after ETC-1922159 treatment of CRC).

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Table 8. 2^{nd} order combinatorial hypotheses between WLS and SNX family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
SNX family w.r.t WLS	
SNX-9/11	WLS (before ETC-1922159 treatment of CRC)
SNX-33	WLS (after ETC-1922159 treatment of CRC)

2.1.5. WLS-ARF

Yu et al. [17] show that WLS Golgi-to-ER retrieval requires the COPI regulator ARF as well as ERGIC2. In colorectal cancer cells treated with ETC-1922159, ARF family and WLS, were found to be up regulated and recorded independently. I was able to rank 2^{nd} order combination of ARF family and WLS, that were up regulated.

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 ARF family w.r.t WLS. ARFGAP3 - WLS shows low ranking of 480 (laplace) and 1075 (rbf). ARF3 - WLS shows low ranking of 866 (laplace), 736 (linear) and 516 (rbf). ARF6 - WLS shows low ranking of 1291 (laplace) and 855 (rbf). ARF1 - WLS shows low ranking of 1526 (laplace) and 1170 (linear). This low ranking points to the fact that the combination is not relevant after ETC-1922159 treatment of CRC, however, it might be prevalent in CRC, before treatment.

Further, ARF4 - WLS shows high ranking of 2040 (laplace) and 2414 (linear). This high ranking points to the fact that the combination is relevant after ETC-1922159 treatment of CRC, however, it might not be prevalent in CRC, before treatment.

Table 9. 2^{nd} order interaction ranking between WLS VS ARF family members.

RANKING ARF FAMILY VS WLS			
RANKING OF ARF FAMILY W.R.T WLS			
	laplace	linear	rbf
ARFGAP3 - WLS	480	2111	1075
ARF3 - WLS	866	736	516
ARF6 - WLS	1291	1974	855
ARF1 - WLS	1526	1170	2079
ARF4 - WLS	2040	2414	1062

One can also interpret the results of the table 9 graphically, with the following influences - • ARF family w.r.t WLS with WLS $->$ ARF-GAP3/3/6/1 (before ETC-1922159 treatment of CRC) and WLS $->$ ARF-4 (after ETC-1922159 treatment of CRC).

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Table 10. 2^{nd} order combinatorial hypotheses between WLS and ARF family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
ARF family w.r.t WLS	
ARF-GAP3/3/6/1	WLS (before ETC-1922159 treatment of CRC)
ARF-4	WLS (after ETC-1922159 treatment of CRC)

2.1.6. WLS-UBE2

Wolf et al. [18] found that EVI/WLS is ubiquitylated and degraded in cells irrespective of their level of WNT production. This ubiquitylation is mediated by the E2 ubiquitin-conjugating enzymes UBE2K, UBE2J2 and UBE2N. In colorectal cancer cells treated with ETC-1922159, UBE2 family and WLS, were found to be up regulated and recorded independently. I was able to rank 2^{nd} order combination of UBE2 family and WLS, that were up regulated.

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 UBE2 family w.r.t WLS. UBE2H - WLS shows low ranking of 868 (laplace), 1409 (linear) and 1051 (rbf). UBE2J1 - WLS shows low ranking of 957 (laplace) and 1346 (linear). UBE2F - WLS shows low ranking of 1379 (laplace), 356 (linear) and 221 (rbf). This low ranking points to the fact that the combination is not relevant after ETC-1922159 treatment of CRC, however, it might be prevalent in CRC, before treatment.

Further, UBE2A - WLS shows high ranking of 1865 (laplace) and 2340 (linear). UBE2Z - WLS shows high ranking of 2032 (laplace) and 2265 (rbf). UBE2B - WLS shows high ranking of 2353 (laplace) and 1925 (rbf). This high ranking points to the fact that the combination is relevant after ETC-1922159 treatment of CRC, however, it might not be prevalent in CRC, before treatment.

Table 11. 2^{nd} order interaction ranking between WLS VS UBE2 family members.

RANKING UBE2 FAMILY VS WLS			
RANKING OF UBE2 FAMILY W.R.T WLS			
	laplace	linear	rbf
UBE2H - WLS	868	1409	1051
UBE2J1 - WLS	957	1346	2165
UBE2F - WLS	1379	356	221
UBE2A - WLS	1865	2340	1213
UBE2Z - WLS	2032	1584	2265
UBE2B - WLS	2353	816	1925

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One can also interpret the results of the table 11 graphically, with the following influences - • UBE2 family w.r.t WLS with WLS $->$ UBE2-H/J1/F (before ETC-1922159 treatment of CRC) and WLS $->$ UBE2-A/Z/B (after ETC-1922159 treatment of CRC).

Table 12. 2^{nd} order combinatorial hypotheses between WLS and UBE2 family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
UBE2 family w.r.t WLS	
UBE2-H/J1/F	WLS (before ETC-1922159 treatment of CRC)
UBE2-A/Z/B	WLS (after ETC-1922159 treatment of CRC)

2.1.7. WLS-ATPases

McGough et al. [19] demonstrate the role of SNX3 for WNTLESS transport and report that SNX3 associates with a membrane remodelling complex composed of MON2, DOPEY2 and the putative aminophospholipid translocase, ATP9A. ATP9A comes under the category of one of the P-type ATPases (under the general category of ATPases). In colorectal cancer cells treated with ETC-1922159, ATP family and WLS, were found to be up regulated and recorded independently. I was able to rank 2^{nd} order combination of ATP family and WLS, that were up regulated.

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 ATP family w.r.t WLS. ATP1B1 - WLS shows low ranking of 302 (laplace) and 241 (rbf). ATP2B4 - WLS shows low ranking of 303 (laplace), 236 (linear) and 335 (rbf). ATP2A2 - WLS shows low ranking of 340 (laplace), 1116 (linear) and 76 (rbf). ATP13A2 - WLS shows low ranking of 657 (laplace), 306 (linear) and 1173 (rbf). ATP2B1 - WLS shows low ranking of 778 (laplace) and 346 (rbf). This low ranking points to the fact that the combination is not relevant after ETC-1922159 treatment of CRC, however, it might be prevalent in CRC, before treatment.

Further, ATP6V1E1 - WLS shows high ranking of 1660 (laplace) and 1924 (rbf). ATP10B - WLS shows high ranking of 1810 (laplace) and 1527 (rbf). ATP6V1D - WLS shows high ranking of 1897 (laplace) and 1807 (linear). ATP11B - WLS shows high ranking of 1929 (laplace), 2336 (linear) and 2421 (rbf). ATP6V0D1 - WLS shows high ranking of 2435 (laplace), 1724 (linear) and 2289 (rbf). This high ranking points to the fact that the combination is relevant after ETC-1922159 treatment of CRC, however, it might not be prevalent in CRC, before treatment.

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Table 13. 2^{nd} order interaction ranking between WLS VS ATP family members.

RANKING ATP FAMILY VS WLS			
RANKING OF ATP FAMILY W.R.T WLS			
	laplace	linear	rbf
ATP1B1 - WLS	302	1601	241
ATP2B4 - WLS	303	236	335
ATP2A2 - WLS	340	1116	76
ATP13A2 - WLS	657	306	1173
ATP2B1 - WLS	778	1558	346
ATP6V1E1 - WLS	1660	1183	1924
ATP10B - WLS	1810	937	1527
ATP6V1D - WLS	1897	1807	65
ATP11B - WLS	1929	2336	2421
ATP6V0D1 - WLS	2435	1724	2289

One can also interpret the results of the table 13 graphically, with the following influences - • ATP family w.r.t WLS with WLS $-$ > ATP-1B1/2B4/2A2/13A2/2B1 (before ETC-1922159 treatment of CRC) and WLS $-$ > ATP-6V1E1/10B/6V1D/11B/6V0D1 (after ETC-1922159 treatment of CRC).

Table 14. 2^{nd} order combinatorial hypotheses between WLS and ATP family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
ATP family w.r.t WLS	
ATP-1B1/2B4/2A2/13A2/2B1	WLS (before ETC-1922159 treatment of CRC)
ATP-6V1E1/10B/6V1D/11B/6V0D1	WLS (after ETC-1922159 treatment of CRC)

2.1.8. WLS-TMEM

Li and Niswander [20] discovered a novel regulator of WNT pathway called TMEM132A. They show physical evidence and functional interaction of TMEM132A with WLS. In colorectal cancer cells treated with ETC-1922159, TMEM family and WLS, were found to be up regulated and recorded independently. I was able to rank 2^{nd} order combination of TMEM family and WLS, that were up regulated.

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 TMEM family w.r.t WLS. TMEM61 - WLS shows low ranking of 141 (laplace), 107 (linear) and 748 (rbf). TMEM45A - WLS shows low ranking of 149 (laplace), 1276 (linear) and 534 (rbf). TMEM120B -

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WLS shows low ranking of 166 (laplace), 53 (linear) and 169 (rbf). TMEM40 - WLS shows low ranking of 172 (laplace), 114 (linear) and 879 (rbf). TMEM86A - WLS shows low ranking of 203 (laplace), 664 (linear) and 614 (rbf). TMEM50B - WLS shows low ranking of 224 (laplace), 1411 (linear) and 464 (rbf). TMEM63B - WLS shows low ranking of 300 (laplace) and 236 (rbf). TMEM171 - WLS shows low ranking of 435 (laplace), 139 (linear) and 484 (rbf). TMEM82 - WLS shows low ranking of 441 (laplace) and 334 (linear). TMEM253 - WLS shows low ranking of 494 (laplace) and 896 (linear). TMEM45B - WLS shows low ranking of 506 (laplace), 14 (linear) and 347 (rbf). TMEM92 - WLS shows low ranking of 672 (laplace), 248 (linear) and 17 (rbf). TMEM127 - WLS shows low ranking of 764 (laplace), 1117 (linear) and 1219 (rbf). TMEM120A - WLS shows low ranking of 1000 (laplace), 868 (linear) and 43 (rbf). TMEM164 - WLS shows low ranking of 1149 (laplace) and 1048 (rbf). TMEM150B - WLS shows low ranking of 1165 (laplace) and 1360 (linear). TMEM106A - WLS shows low ranking of 1166 (laplace), 162 (linear) and 1095 (rbf). TMEM62 - WLS shows low ranking of 1173 (laplace) and 796 (rbf). TMEM159 - WLS shows low ranking of 1205 (laplace) and 1551 (rbf). TMEM139 - WLS shows low ranking of 1235 (laplace) and 1127 (linear). TMEM54 - WLS shows low ranking of 1302 (laplace), 1080 (linear) and 1371 (rbf). TMEM217 - WLS shows low ranking of 1432 (laplace), 530 (linear) and 291 (rbf). TMEM176B - WLS shows low ranking of 892 (linear) and 313 (rbf). TMEM229B - WLS shows low ranking of 1392 (linear) and 83 (rbf). TMEM51-AS1 - WLS shows low ranking of 1389 (linear) and 1109 (rbf). TMEM8A - WLS shows low ranking of 832 (linear) and 1151 (rbf). This low ranking points to the fact that the combination is not relevant after ETC-1922159 treatment of CRC, however, it might be prevalent in CRC, before treatment.

Further, TMEM79 - WLS shows high ranking of 2278 (linear) and 1822 (rbf). TMEM184A - WLS shows high ranking of 1545 (laplace) and 2070 (rbf). TMEM65 - WLS shows high ranking of 1581 (laplace) and 2121 (linear). TMEM185A - WLS shows high ranking of 1666 (laplace) and 1561 (linear). TMEM234 - WLS shows high ranking of 1736 (laplace) and 1678 (rbf). TMEM57 - WLS shows high ranking of 2005 (laplace), 2106 (linear) and 2254 (rbf). TMEM44 - WLS shows high ranking of 2179 (laplace), 2154 (linear) and 2454 (rbf). TMEM176A - WLS shows high ranking of 2255 (laplace), 1699 (linear) and 1588 (rbf). TMEM220 - WLS shows high ranking of 2292 (laplace), 2468 (linear) and 2428 (rbf). TMEM30B - WLS shows high ranking of 2297 (laplace) and 2108 (rbf). TMEM184B - WLS shows high ranking of 2308 (laplace), 2381 (linear) and 1706 (rbf). TMEM140 - WLS shows high ranking of 2342 (laplace), 1862 (linear) and 2347 (rbf). TMEM2 - WLS shows high ranking of 2472 (laplace), 1865 (linear) and 1978 (rbf). TMEM31 - WLS shows high ranking of 2513 (laplace), 2481 (linear) and 2305 (rbf). This high ranking points to the fact that the combination is relevant after ETC-1922159 treatment of CRC, however, it might not be prevalent in CRC, before treatment.

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Table 15. 2^{nd} order interaction ranking between WLS VS TMEM family members.

RANKING TMEM FAMILY VS WLS							
RANKING OF TMEM FAMILY W.R.T WLS							
	laplace	linear	rbf		laplace	linear	rbf
TMEM61 - WLS	141	107	748	TMEM45A - WLS	149	1276	534
TMEM120B - WLS	166	53	169	TMEM40 - WLS	172	114	879
TMEM86A - WLS	203	664	614	TMEM50B - WLS	224	1411	464
TMEM63B - WLS	300	1853	236	TMEM171 - WLS	435	139	484
TMEM82 - WLS	441	334	1748	TMEM253 - WLS	494	896	1976
TMEM45B - WLS	506	14	347	TMEM79 - WLS	555	2278	1822
TMEM92 - WLS	672	248	17	TMEM127 - WLS	764	1117	1219
TMEM120A - WLS	1000	868	43	TMEM164 - WLS	1149	2170	1048
TMEM150B - WLS	1165	1360	2389	TMEM106A - WLS	1166	162	1095
TMEM62 - WLS	1173	2339	796	TMEM159 - WLS	1205	2159	1551
TMEM139 - WLS	1235	1127	1742	TMEM54 - WLS	1302	1080	1371
TMEM217 - WLS	1432	530	291	TMEM184A - WLS	1545	729	2070
TMEM65 - WLS	1581	2121	919	TMEM176B - WLS	1624	892	313
TMEM185A - WLS	1666	1561	873	TMEM229B - WLS	1670	1392	83
TMEM234 - WLS	1736	443	1678	TMEM57 - WLS	2005	2106	2254
TMEM44 - WLS	2179	2154	2454	TMEM176A - WLS	2255	1699	1588
TMEM51-AS1 - WLS	2261	1389	1109	TMEM220 - WLS	2292	2468	2428
TMEM30B - WLS	2297	1378	2108	TMEM184B - WLS	2308	2381	1706
TMEM8A - WLS	2310	832	1151	TMEM140 - WLS	2342	1862	2347
TMEM2 - WLS	2472	1865	1978	TMEM31 - WLS	2513	2481	2305

One can also interpret the results of the table 15 graphically, with the following influences - • TMEM family w.r.t WLS with WLS $->$ TMEM-61 / 45A / 120B / 40 / 86A / 50B / 63B / 171 / 82 / 253 / 45B / 92 / 127 / 120A / 164 / 150B / 106A / 62 / 159 / 139 / 54 / 217 / 176B / 229B / 51-AS1 / 8A (before ETC-1922159 treatment of CRC) and WLS $->$ TMEM-79 / 184A / 65 / 185A / 234 / 57 / 44 / 176A / 220 / 30B / 184B / 140 / 2 / 31 (after ETC-1922159 treatment of CRC).

Table 16. 2^{nd} order combinatorial hypotheses between WLS and TMEM family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
TMEM family w.r.t WLS	
BEFORE ETC-1922159 TREATMENT OF CRC	
TMEM-61/45A/120B/40/86A/50B/63B/171/82/253/45B	WLS
TMEM-92/127/120A/164/150B/106A/62/159/139/54	WLS
TMEM-217/176B/229B/51-AS1/8A	WLS
AFTER ETC-1922159 TREATMENT OF CRC	
TMEM-79/184A/65/185A/234/57/44/176A/220/30B	WLS
TMEM-184B/140/2/31	WLS

3. Conclusion

Presented here are a range of multiple synergistic WLS 2^{nd} order combinations that were ranked via a machine learning based search engine. Via majority voting across the ranking methods, it was

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possible to find plausible unexplored synergistic combinations of WLS-X that might be prevalent in CRC cells after treatment with ETC-1922159 drug.

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Data Availability Statement: Data used in this research work was released in a publication in Madan et al. [21].

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