Machine Learning Discoveries of ATG3-X Synergy in etc-1922159 Treated Colorectal Cancer Cells

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Article

Machine Learning Discoveries of ATG3-X Synergy in ETC-1922159 Treated Colorectal Cancer Cells †

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† ML dicoveries of ATG3-X synergy in ETC-1922159 treated CRC cells

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Abstract: Autophagy related 3 (ATG3) is one of the genes that plays a major role in autophagy. It has been found to be highly expressed in colon caner. In colorectal cancer (CRC) cells treated with ETC-1922159, ATG3 was found to be down regulated along with other genes. A recently developed search engine ranked combinations of ATG3-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, there are other that have yet to be explored or tested. These rankings reveal which ATG3-X combinations might be working synergistically in CRC. In this research work, I cover combinations of ATG3 with programmed cell death (PDCD), bromodomain containing (BRD), aspartyl-tRNA synthetase (DARS), methyltransferase N6-adenosine-methyltransferase non-catalytic subunit (METTL), eukaryotic translation initiation factor (EIF), forkhead box (FOX), growth arrest specific (GAS), RAB member RAS oncogene GTPases (RAB), FA complementation groups (RAD51), tripartite motif containing (TRIM) and TNF alpha induced protein (TNFAIP) family.

Keywords: ATG3; Porcupine inhibitor ETC-1922159; sensitivity analysis; colorectal cancer

1. Introduction

1.1. Autophagy

Authophagy is a natural process in which cells works towards degradation of dysfunctional proteins and other cytoplasmic cargo via lysosome dependent mechanism. Hitherto, there are three ways by which autophagy happens - (1) macroautopahgy, (2) chaperone mediated autophagy and (3) microautophagy. Out of these the first one is the most widely researched topic. Autophagy was coined by Christian de Duve in 1963, after his discovery of the exitence of lysosomes which were involved in the process (De Duve and Wattiaux [1] and Klionsky [2]). Later, Takeshige et al. [3] first observed the autophagy degradation in yeast cells. This was followed by isolation and characterization of autophagy causing genes in Tsukada and Ohsumi [4]. Introductory reviews on autophagy can be found in Levine and Kroemer [5] and Levine and Kroemer [6]. The reviews also cover the roles of autophagy genes in cancer, briefly. A recent study by Li et al. [7], discusses the role of autophagy and its related genes (i.e ATGs) in both cancer suppression as well as cancer promotion.

1.2. ATG3

ATG3 is a protein that lacks rigid structure and more specifically it is a ubiquitin carrier protein E2-like enzyme. The crystal structure of ATG3 has been elucidated in Yamada et al. [8]. ATG3 engages with many binding partners and binding sites. Fang et al. [9] indicate that ATG3 interacts with ATG7 via formation of an E1-E2 complex, LC3/ATG8 via a thioester bond and ATG12 via leucine of the LC3-interacting region motif, while providing necessary references for the same. They also cite references which show that ATG3 is implicated in various types of cancers. In a recent finding, Huang et al. [10] show that ATG3 promotes colon cancer. ATG3 was found to be down regulated in colorectal cancer cell lines after the treatment of ETC-1922159 drug as observed in Madan et al.

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[11]. Most studies till now, have dealt with how the ATG3 works and there is very less informantion reagarding which gene/protein combinations might be working synergistically along with it. It would be nice to observe if there is any connection between the independently observed factors in the form of unknown biological hypotheses. To solve the issue, the next section discusses a solution to the problem.

1.3. Combinatorial search problem and a possible solution

In a recently published work Sinha [12], 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 [13]. The work uses SVM package by Joachims [14] 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. ATG3 related synergies

2.1.1. ATG3-PDCD

Murrow et al. [15] identify an interaction between ATG12-ATG3 and PDCD6IP and demonstrate that the interaction controls multiple PDCD6IP dependent processes like exosome biogenesis, late endosome distribution,, and viral budding. In colorectal cancer cells treated with ETC-1922159, PDCD family members and ATG3, were found to be down regulated and recorded independently. I was able to rank 2^{nd} order combinations of PDCD family members and ATG3, that were down 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 PDCD family w.r.t ATG3. PDCD2 - ATG3 shows low ranking of 338 (laplace) and 272 (linear). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, PDCD2L, PDCD11 and PDCD7 showed high ranking with ATG3, thus indicating that they might not be working synergistically with ATG3, before the drug treatment.

Table 1. 2^{nd} order interaction ranking between ATG3 VS PDCD family members.

RANKING PDCD FAMILY VS ATG3			
RANKING OF PDCD FAMILY W.R.T ATG3			
	laplace	linear	rbf
PDCD2 - ATG3	338	272	1974
PDCD2L - ATG3	643	1890	1069
PDCD11 - ATG3	1683	2573	1307
PDCD7 - ATG3	2706	1722	1648

One can also interpret the results of the Table 1 graphically, with the following influences - • PDCD family w.r.t ATG3 with ATG3 $-$ > PDCD2.

Table 2. 2^{nd} order combinatorial hypotheses between ATG3 and PDCD family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
PDCD family w.r.t ATG3	
PDCD2	ATG3

2.1.2. ATG3-BRD

In acute myelogenous leukemia cells, Huang et al. [16] found that BRD4 binds to the promoters of ATG3, and expression of this gene is reduced by inhibitors of BRD4. Thus BRD4 plays a direct role in

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autophagy by regulating the transcription of ATG3. In colorectal cancer cells treated with ETC-1922159, BRD family members and ATG3, were found to be down regulated and recorded independently. I was able to rank 2^{nd} order combinations of BRD family members and ATG3, that were down 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 BRD family w.r.t ATG3. BRD8 - ATG3 shows low ranking of 453 (laplace), 574 (linear) and 1550 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Table 3. 2^{nd} order interaction ranking between ATG3 VS BRD family members.

RANKING BRD FAMILY VS ATG3			
	RANKING OF BRD FAMILY W.R.T ATG3		
	laplace	linear	rbf
BRD8 - ATG3	453	574	1550

One can also interpret the results of the Table 3 graphically, with the following influences - • BRD family w.r.t ATG3 with ATG3 $-$ $>$ BRD8.

Table 4. 2^{nd} order combinatorial hypotheses between ATG3 and BRD family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
BRD family w.r.t ATG3	
BRD8	ATG3

2.1.3. ATG3-DARS/METTL

In cervical cancer (CC), Shen et al. [17] experimentally confirmed that DARS-AS1 regulated the expression of ATG3 to affect CC cell autophagy by modulating DARS expression. Further, they show that DARS-AS1 recruits METTL3 and METTL14 mediated m^6A methylation to translate DARS translation. In colorectal cancer cells treated with ETC-1922159, DARS (and METTL) family members and ATG3, were found to be down regulated and recorded independently. I was able to rank 2^{nd} order combinations of DARS (and METTL) family members and ATG3, that were down 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 DARS family w.r.t ATG3. DARS2 - ATG3 shows low ranking of 1242 (laplace), 1451 (linear) and 512 (rbf). METTL1 - ATG3 shows low ranking of 472 (laplace), 337 (linear) and 391 (rbf). METTL8 - ATG3 shows low ranking of 1287 (laplace) and 1469 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, METTL13, METTL3, METTL21A, METTL17, METTL16, METTL5, METTL12, METTL21B and METTL2B showed high ranking with ATG3, thus indicating that they might not be working synergistically with ATG3, before the drug treatment.

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Table 5. 2^{nd} order interaction ranking between ATG3 VS DARS family members.

RANKING DARS (AND METTL) FAMILY VS ATG3			
RANKING OF DARS (AND METTL) FAMILY W.R.T ATG3			
	laplace	linear	rbf
DARS2 - ATG3	1242	1451	512
METTL1 - ATG3	472	337	391
METTL8 - ATG3	1287	1971	1469
METTL13 - ATG3	1515	2540	1811
METTL3 - ATG3	1681	2358	2070
METTL21A - ATG3	1817	2034	1709
METTL17 - ATG3	1990	2695	2157
METTL16 - ATG3	2125	2260	1061
METTL5 - ATG3	2166	2530	2039
METTL12 - ATG3	2222	1756	1772
METTL21B - ATG3	2403	2003	2366
METTL2B - ATG3	2436	2322	1941

One can also interpret the results of the Table 5 graphically, with the following influences - • DARS family w.r.t ATG3 with ATG3 $->$ DARS2 and ATG3 $->$ METTL-1/8.

Table 6. 2^{nd} order combinatorial hypotheses between ATG3 and DARS family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
DARS (and METTL) family w.r.t ATG3	
DARS2	ATG3
METTL-1/8	ATG3

2.1.4. ATG3-EIF

EIF5A function is well-described in yeast and bacteria, but little is known about its translational targets in human cells. Frankel [18] using liquid chromatography-mass spectrometry (LC-MS) analysis, revealed that EIF5A affects the translation of ATG3. It was confirmed that ATG3 protein levels were reduced upon knockdown of EIF5A. In colorectal cancer cells treated with ETC-1922159, EIF family members and ATG3, were found to be down regulated and recorded independently. I was able to rank 2^{nd} order combinations of EIF family members and ATG3, that were down 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 EIF family w.r.t ATG3. EIF2B1 - ATG3 shows low ranking of 784 (laplace) and 485 (linear). EIF2D - ATG3 shows low ranking of 1300 (laplace), 1108 (linear) and 1497 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, EIF2B3, EIF3F, EIF3L, EIF3E, EIF2AK4, EIF4B, EIF2B5 and EIF4EBP1 showed high ranking with ATG3, thus indicating that they might not be working synergistically with ATG3, before the drug treatment.

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Table 7. 2^{nd} order interaction ranking between ATG3 VS EIF family members.

RANKING EIF FAMILY VS ATG3							
	RANKING OF EIF FAMILY W.R.T ATG3						
	laplace	linear	rbf		laplace	linear	rbf
EIF2B1 - ATG3	784	485	2014	EIF2D - ATG3	1300	1108	1497
EIF2B3 - ATG3	1601	1947	1165	EIF3F - ATG3	1826	2612	1861
EIF3L - ATG3	1883	1801	2185	EIF3E - ATG3	1936	2142	1131
EIF2AK4 - ATG3	2099	2654	2052	EIF4B - ATG3	2270	2577	1524
EIF2B5 - ATG3	2614	2678	2387	EIF4EBP1 - ATG3	2729	579	2622

One can also interpret the results of the Table 7 graphically, with the following influences - • EIF family w.r.t ATG3 with ATG3 $-$ > EIF-2B1/2D.

Table 8. 2^{nd} order combinatorial hypotheses between ATG3 and EIF family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
EIF family w.r.t ATG3	
EIF-2B1/2D	ATG3

2.1.5. ATG3-FOX

In hypoxic granulosa cells, Li et al. [19] show that members of forkhead box proteins FOXO, stimulate the upregulation of ATG3. In colorectal cancer cells treated with ETC-1922159, FOX family members and ATG3, were found to be down regulated and recorded independently. I was able to rank 2^{nd} order combinations of FOX family members and ATG3, that were down 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 FOX family w.r.t ATG3. FOXM1 - ATG3 shows low ranking of 14 (laplace), 66 (linear) and 147 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, FOXD2-AS1, FOXA2 and FOXJ1 showed high ranking with ATG3, thus indicating that they might not be working synergistically with ATG3, before the drug treatment.

Table 9. 2^{nd} order interaction ranking between ATG3 VS FOX family members.

RANKING FOX FAMILY VS ATG3			
	RANKING OF FOX FAMILY W.R.T ATG3		
	laplace	linear	rbf
FOXM1 - ATG3	14	66	147
FOXD2-AS1 - ATG3	1667	1571	2633
FOXA2 - ATG3	1946	1898	1198
FOXJ1 - ATG3	2711	2644	2740

One can also interpret the results of the Table 9 graphically, with the following influences - • FOX family w.r.t ATG3 with ATG3 $-$ > FOXM1.

Table 10. 2^{nd} order combinatorial hypotheses between ATG3 and FOX family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
FOX family w.r.t ATG3	
FOXM1	ATG3

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2.1.6. ATG3-GAS

Li et al. [20] showed that knockdown of GAS5 suppressed the expression of LC3II, ATG3 and ATG5-ATG12 complex formation, thus suggesting that GAS5/miR-23a/ATG3 axis might be a regulatory network contributing to autophagy and cell viability. In colorectal cancer cells treated with ETC-1922159, GAS family members and ATG3, were found to be down regulated and recorded independently. I was able to rank 2^{nd} order combinations of GAS family members and ATG3, that were down regulated.

The Table 11 shows rankings of GAS family w.r.t ATG3. Interestingly, all recorded family members, i.e GAS5, GAS5-AS1, GAS2L3, GAS6 and GAS6-AS1 showed high ranking with ATG3, thus indicating that they might not be working synergistically with ATG3, before the drug treatment.

Table 11. 2^{nd} order interaction ranking between ATG3 VS GAS family members.

RANKING GAS FAMILY VS ATG3			
RANKING OF GAS FAMILY W.R.T ATG3			
	laplace	linear	rbf
GAS5 - ATG3	954	2092	1870
GAS5-AS1 - ATG3	1607	2016	1841
GAS2L3 - ATG3	2354	1931	2264
GAS6 - ATG3	2491	1407	2328
GAS6-AS1 - ATG3	2604	1368	2705

2.1.7. ATG3-RAB

ATG16L facilitates LC3/ATG8-conjugation to phos-phatidylethanolamine by forming a complex with ATG12-conjugated ATG5 and recruiting an LC3-ATG3 intermediate to elongating isolation membranes. Fukuda and Itoh [21] report that ATG16L interacts with the Golgi-resident small GTPase RAB33B and RAB33A. Thus there exists a synergy or connection between RAB33-A/B and ATG3. In colorectal cancer cells treated with ETC-1922159, RAB family members and ATG3, were found to be down regulated and recorded independently. I was able to rank 2^{nd} order combinations of RAB family members and ATG3, that were down regulated.

Table 12 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in Table 13 generated from analysis of the ranks in Table 12. The Table 12 shows rankings of RAB family w.r.t ATG3. RABEPK - ATG3 shows low ranking of 634 (laplace) and 1261 (rbf). RAB26 - ATG3 shows low ranking of 851 (laplace), 1180 (linear) and 157 (rbf). RAB23 - ATG3 shows low ranking of 1121 (laplace) and 1073 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, RAB40B, RAB11FIP3, RABL5, RAB36 and RAB40A showed high ranking with ATG3, thus indicating that they might not be working synergistically with ATG3, before the drug treatment.

Table 12. 2^{nd} order interaction ranking between ATG3 VS RAB family members.

RANKING RAB FAMILY VS ATG3			
RANKING OF RAB FAMILY W.R.T ATG3			
	laplace	linear	rbf
RABEPK - ATG3	634	1626	1261
RAB26 - ATG3	851	1180	157
RAB23 - ATG3	1121	2057	1073
RAB40B - ATG3	2007	2584	1810
RAB11FIP3 - ATG3	2117	2672	2106
RABL5 - ATG3	2353	1197	2241
RAB36 - ATG3	2521	1510	2598
RAB40A - ATG3	2664	2242	2629

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One can also interpret the results of the Table 12 graphically, with the following influences - • RAB family w.r.t ATG3 with ATG3 $-$ > RAB-EPK/26/23.

Table 13. 2^{nd} order combinatorial hypotheses between ATG3 and RAB family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
RAB family w.r.t ATG3	
RAB-EPK/26/23	ATG3

2.1.8. ATG3-RAD51

RAD51 plays a major role in homologous recombination but it is unclear whether RAD51 can be involved in gene regulation as a co-factor. Kang et al. [22] show results which suggest that RAD51 contributes to the regulation of autophagy-related genes like ATG3 and ATG5 in a DNA-binding-dependent manner. In colorectal cancer cells treated with ETC-1922159, RAD51 family members and ATG3, were found to be down regulated and recorded independently. I was able to rank 2^{nd} order combinations of RAD51 family members and ATG3, that were down regulated.

Table 14 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in Table 15 generated from analysis of the ranks in Table 14. The Table 14 shows rankings of RAD51 family w.r.t ATG3. RAD51 - ATG3 shows low ranking of 142 (laplace), 1039 (linear) and 642 (rbf). RAD51C - ATG3 shows low ranking of 757 (laplace), 1200 (linear) and 291 (rbf). RAD51AP1 - ATG3 shows low ranking of 67 (laplace), 644 (linear) and 385 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Table 14. 2^{nd} order interaction ranking between ATG3 VS RAD51 family members.

RANKING RAD51 FAMILY VS ATG3			
RANKING OF RAD51 FAMILY W.R.T ATG3			
	laplace	linear	rbf
RAD51 - ATG3	142	1039	642
RAD51C - ATG3	757	1200	291
RAD51AP1 - ATG3	67	644	385

One can also interpret the results of the Table 14 graphically, with the following influences - • RAD51 family w.r.t ATG3 with ATG3 $-$ > RAD-51/51C/51AP1.

Table 15. 2^{nd} order combinatorial hypotheses between ATG3 and RAD51 family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
RAD51 family w.r.t ATG3	
RAD-51/51C/51AP1	ATG3

2.1.9. ATG3-TRIM

After L. monocytogenes infection, Wang et al. [23] found that TRIM7 overexpression resulted in enhanced LC3-ATG3 association, thus implicating TRIM7 as a regulator for autophagy. In colorectal cancer cells treated with ETC-1922159, TRIM family members and ATG3, were found to be down regulated and recorded independently. I was able to rank 2^{nd} order combinations of TRIM family members and ATG3, that were down regulated.

Table 16 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in Table 17 generated from analysis of the ranks in Table 16. The Table 16 shows rankings of TRIM family w.r.t ATG3. TRIM59 - ATG3 shows low ranking of 1061 (laplace), 458 (linear) and 317 (rbf). TRIM28 - ATG3 shows low ranking of 1351 (laplace) and 727 (linear). These rankings point to

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the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, TRIM32, TRIM7 and TRIM65 showed high ranking with ATG3, thus indicating that they might not be working synergistically with ATG3, before the drug treatment.

Table 16. 2^{nd} order interaction ranking between ATG3 VS TRIM family members.

RANKING TRIM FAMILY VS ATG3			
RANKING OF TRIM FAMILY W.R.T ATG3	laplace	linear	rbf
TRIM59 - ATG3	1061	458	317
TRIM28 - ATG3	1351	727	2434
TRIM32 - ATG3	1898	1733	1808
TRIM7 - ATG3	2095	265	2165
TRIM65 - ATG3	2499	2484	1623

One can also interpret the results of the Table 16 graphically, with the following influences - • TRIM family w.r.t ATG3 with ATG3 $-$ > TRIM-59/28.

Table 17. 2^{nd} order combinatorial hypotheses between ATG3 and TRIM family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
TRIM family w.r.t ATG3	
TRIM-59/28	ATG3

2.1.10. ATG3-TNFAIP

TNFAIP8 regulates autophagy by interacting with ATG3-ATG7 autophagosome complex proteins and promotes hepatocellular carcinoma cell proliferation, as shown by Niture et al. [24]. Thus these is a direct connection between TNFAIP8 and ATG3. In colorectal cancer cells treated with ETC-1922159, TNFAIP family members and ATG3, were found to be down regulated and recorded independently. I was able to rank 2^{nd} order combinations of TNFAIP family members and ATG3, that were down regulated.

Table 18 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in Table 19 generated from analysis of the ranks in Table 18. The Table 18 shows rankings of TNFAIP family w.r.t ATG3. TNFAIP8L1 - ATG3 shows low ranking of 781 (laplace) and 126 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Table 18. 2^{nd} order interaction ranking between ATG3 VS TNFAIP family members.

RANKING TNFAIP FAMILY VS ATG3			
RANKING OF TNFAIP FAMILY W.R.T ATG3	laplace	linear	rbf
TNFAIP8L1 - ATG3	781	1702	126

One can also interpret the results of the Table 18 graphically, with the following influences - • TNFAIP family w.r.t ATG3 with ATG3 $-$ > TNFAIP8L1.

Table 19. 2^{nd} order combinatorial hypotheses between ATG3 and TNFAIP family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
TNFAIP family w.r.t ATG3	
TNFAIP8L1	ATG3

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3. Conclusion

Presented here are a range of multiple synergistic ATG3 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 ATG3-X that might be prevalent in CRC cells after treatment with ETC-1922159 drug.

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

Source of Data: Data used in this research work was released in a publication in Madan et al. [11].

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