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Research article

Predicting, designing, characterization and evaluation of a new novel anticancer peptide SSVAM-9 against the lung carcinoma, an *insilico* approach

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ABSTRACT

Several anticancer drugs are getting resisted by the cancer cell and treatment like chemotherapy, radiation causes serious side effects. In immunomodulatory treatment the efficiency is less and CAR-T cells, CAR-NK cells require enormous time to get adopt to the *in vitro* and may cause seizures, dilemma, concussion in prolonged use against the cancer. Even the production of CAR-T cells and NK cells are tedious process. To overcome this situation, anticancer peptides can be used, as they don't have any drug resistance and they can be highly potent, with good cell penetration. The advantages of these peptides are easy to modify, produce and formulate. This pandemic showed us that, identifying and characterizing a novel anticancer peptide (ACP) is an extremely time and labor consuming process. To reduce the time and labor, this study uses several *in silico* tools and algorithms like SVM, RF, XGBoost and KNN to predict a novel anticancer peptide. After several studies, with the collected data, a novel anticancer peptide – SSVAM-9 was predicted, which acts against the lung carcinoma. In this, anticancer activity prediction, cell permeation prediction with all 4 algorithms; stability prediction, allergenicity prediction and activity on lung carcinoma prediction were carried out in *in silico* model. Considering all the parameters, one best novel ACP was selected (SSVAM-9), and it can be easily formulated as the peptide is a stable one. This approach is an advantageous one as it is cost efficient and less-time consuming which can be studied *in vivo* and *in vitro* in future.

Keywords: Anticancer peptides, Machine Learning, Lung carcinoma, A549, *Insilico.*

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INTRODUCTION

Carcinoma is defined as uncontrollable growth in cells, driven by the mutated genetic material. Carcinoma is one of the greatest challenges for scientists and researchers to identify and develop new drugs with less toxicity and higher efficiency [1]. A benign tumor is easy to treat but when the local cancer cell travels to the other organs of the human body it becomes a malignant tumor which is very difficult to treat. Less than 5% of cancer cases are due to gene mutation and the rest of the cases are due to environmental factors and lifestyle [2]. One of the most common cancers in the global population is lung carcinoma. Tobacco is one of the main causes of 90 percent of lung cancer cases. The rest of the cases are due to environmental factors like the carcinogenic substances present in the air pollutions. Drugs which are used to treat cancer are more toxic, even though there are no other choices of less toxic drugs to treat cancer. Even chemotherapy can cause more side effects [3]. Immunomodulatory treatment has very less efficiency than chemotherapy. Radiation can be used to treat cancer, but it causes damage to the human immune cells and other cells also. Newly discovered CAR-T and CAR-NK are chimeric antigen receptor gene-modified cells having a good anticancer effect in *in vivo* studies. But when it is given *in vitro*, the efficiency is less at the starting level and causes serious side effects as it starts to release excess cytokines due to overreacting with the cancer cells. Due to the complex process, the training with the cancer cells and production of the CAR-T and CAR-NK cells became a huge obstacle for the treatment [4,5]. This creates a research gap for scientists and researchers to find an alternative treatment to treat or cure cancer. One of the booming fields to treat cancer in an alternative method is anticancer peptides. The anticancer peptide can be at any length of 10-60 amino acids. They have less or no drug resistance and are easily degraded by the enzymes in the body [6]. This study aims to identify new anticancer peptides (ACPs) which should be stable, non-allergic, high potent, less toxic with good

cell penetration. This study uses several *in-silico* tools, algorithms like Support vector machines (SVM), Random Forest (RF), eXtreme Gradient Boost (XGBoost), and KNN to create an in-silico model to predict, identify the ACP against lung carcinoma which can be further evaluated in *in vivo* and *in vitro* in future.

MATERIALS AND METHODS

Procurement of the ACP against A549

Temporin-Las crdd.osdd.net/raghava/cancerppd/ display_ sub. php?details=1691), an ACP of linear sequence length 13, which have an activity against lung cancer has been taken as reference from CancerPPD for the upcoming prediction. Several other features like C-terminal modification, N-terminal modification, and the percentage of the activity were considered [7]. With the help of this peptide and ProtParam parameters (web.expasy.org/ protparam/), we are predicting a new peptide that can further be better in activity against lung cancer than this peptide, more stable and non-allergic when it is infused the humans [8].

Uncovering homogeneous peptides with blastp

The peptide sequence "LLRHVVKILSKYL" was blasted against the proteins database in NCBI with the blastpsuite (blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=Blastp & PAGE_ TYPE=BlastSearch&LINK_LOC=blasthome) and the similar homogenous proteins were scrutinized [9]. From the protein sequence, a similar peptide sequence was manually picked up and was selected for further study.

Prognosticating the ACP activity of the scrutinized peptide sequence

The anticancer activity of the scrutinized peptide was examined with the CpACpP web server (http://cbb1.ut.ac.ir/ CpACpP/Index) with three algorithms – XGBoost (eXtreme Gradient Boosting), SVM (Support Vector Machine), and RF (Random Forest) [10]. Only if the peptide passes all the three algorithms, it is selected for the next prediction algorithm – KNN (k-nearest neighbors), which is a self-written program showing the result in the graph.

Dataset devising and prognosticating the ACP activity

The ACP reference peptide sequences were obtained from the CancerPPD(crdd.osdd. net/raghava/cancerppd/). The peptides were carried out for the feature extraction – AAC with the propy3 (pypi.org/project/propy3/) and the data frames arrangement, duplicates were removed with the help of pandas (pandas.pydata.org/) [11]. Then the dimensions of features were reduced with the scikit-learn (scikit-learn.org/stable/) [12]. Then the dataset was prepared, and the activity of the sequence can be predicted.The program was written to take the dataset as the reference scale and the input can be taken with the program. The program automatically converts the sequence into features with the propy3. The features are classified into inputs and the activity of the sequence was predicted with the KNN algorithm to identify whether the sequence has the ACP activity or not.

Mutating the predicted sequence

The predicted sequence has been mutated manually with a single amino acid in a different position without changing the length of the peptide sequence. These peptide sequences have also been selected for predicting the anticancer activity in the CpACpP online tool (cbb1.ut.ac.ir/CpACpP/Index). In addition to the prediction of the ACP activity, the CpACpP online tool also predicts the cell-penetrating peptides. The anticancer peptides which also have good cell-penetrating capability were selected. The reason behind mutating the predicted sequence is to increase the maximum possibility of obtaining the higher efficiency anticancer peptide and it also creates a database of predicted peptide lineage which may be helpful for researchers, to analyze these peptides *invivo* and *invitro*. From the mutated lineage, one best anticancer peptide will be selected based on the upcoming study report.

Analysis of predicted peptide and their mutant lineage

In addition to the above criteria, these anticancer peptides were subjected to additional scrutiny which includes the following criteria also. They are stability of the peptide and non-allergen to the human. Stability is an essential edict to maintain the nature and activity of the peptide or in other terms, denaturation can cause the decrease or nullify the effect of the anticancer peptide.

On other hand, ACP should be non-allergic to humans. Any allergy-causing peptide will have no greater significance and it may also cause an anaphylactic reaction. To prevent this only the ACPs which are both stable and non-allergen were considered. The stability studies were carried out with the ProtParam web server(web.expasy.org/protparam/) and allergic properties of the ACPs were studied in the AllerTop v2.0 webserver (ddg-pharmfac.net/AllerTOP/) [11]. Only the scrutinized ACPs were subjected to the next study.

Prediction of activity against A549 cell line

Temporin-Las (Blasted ACP), the predicted ACP is named as SSVAP-3 and the best predicted ACP from the mutant lineage of the SSVAP-3 is named as SSVAM-9 were evaluated for the intensity of anticancer activity against the lung carcinoma (A549) cell line with the assist of the xDeep-AcPEP (github.com/chen709 847237/xDeep-AcPEP)**[12]**. By comparing the activity of the original ACP sequence with the SSVAP-3 and SSVAM-9 sequences, the intensity of the anticancer activity against the lung carcinoma can be identified. To increase the accuracy of the prediction level, the original anticancer peptide, SSVAP-3, and SSVAM-9 were also evaluated by comparing the graph predicted with the KNN algorithm.

RESULTS AND DISCUSSION

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Temporin-Las, ACP against A549

From the CancerPPD, an anticancer peptide - Temporin-Las (PMID: 22641352) was selected. This is peptide has a length of 13 amino acids with an activity of 50.64% inhibition ratio at 50 µg/ml.

The amino acid composition, molecular weight, theoretical pI, C-terminal modification, N-terminal modification, aliphatic index, instability index, and stability were given in Table 1

Diagnosing a similar sequence with blastp

A sequence like the Temporin-Las was searched with the NCBI protein blast to get several homogeneous sequences. From the several similar sequences, a hypothetical protein ENM84_0125 (Ignisphaera aggregans) (Accession no: HHP81285) was selected. From the obtained protein sequence, a similar Temporin-Las peptide was selected as the predicted sequence which is mentioned in Table 2.

Table 1: Shows the parameters of the Temporin-Las sequence but the sequence is unstable which creates a requirement for an alternative sequence to produce a stable anticancer formulation.

Temporin-Las (PMID: 22641352) – ANTICANCER PEPTIDE	
Sequence	LLRHVVKILSKYL
Activity	50.64% inhibition ratio at 50 µg/ml
Linear/ Cyclic	Linear
L/D/Mix	L
C-ter modification	Free
N-ter modification	Free
Cell line	A549
Amino acid composition	H - 7.7%, I - 7.7%, L - 30.8%, K -15.4%
Molecular weight	1582.01
Theoretical pI	10.29
Aliphatic index	194.62
Instability index	44.93
Stable / Unstable	Unstable

Table 2: Shows the homogeneous sequence procured from the NCBI protein blast and the peptide sequence selected from the protein sequence is highlighted. Both the original peptide sequence and the predicted peptide sequence have the same length of amino acids.

Temporin-Las peptide sequence	LLRHVVKILSKYL
NCBI blasted protein sequence	MQKDYYHQIIDDSYTKDIVKRIRVLFHIEDLVELHINEIDVQEIMLRIDGLAEDNININKVVVYGEEVFIRCGYNEYYE DVEISFKCNERKGYTLPEIYRTLGIR**LAKNVVKILSKYL**SYNREYLIMVLDNGDSIVLEGFRESITFPIIANLMFIAHTHP KTLQPIFSRKDLITTLEILSKRGIGSCVVSSTSLCILRKKPLTLDDYEKFEYLINSVEVATKDVLDVVGFESLYTLNL
Predicted sequence	LAKNVVKILSKYL

Prediction of ACP and CPP activity of the predicted sequence

To evaluate CpACpP online tool, the anticancer peptide (Temporin-Las) was first checked for activity prediction. Only after confirming the result as an anticancer peptide for the Temporin-Las sequence, the predicted sequence was evaluated for the anticancer activity in the CpACpP online tool with SVM, RF, and the XGBoost algorithm. One of the important aspects which have to be considered for the anticancer peptide is cell penetration. The anticancer peptide must also be a cell-penetrating peptide for the high anticancer activity potential. So, it was also predicted with the CpACpP online tool for both sequences. The results of the above-mentioned parametric results were given in Table 3. These sequences will be further evaluated with the KNN algorithm with our program, after the dataset preparation to increase the accuracy of prediction in this *in silico* model.

Prepared dataset and prediction of ACP activity with KNN algorithm

150 manually chosen random anticancer peptides were obtained from the CancerPPD database were marked as the reference for the activity prediction program. The whole sequence amino acid composition was calculated with the propy3 module, and the activity of the sequences was obtained. The features dimension was reduced with the help of a linear discriminant analysis module in the scikit-learn package. Then the program with the KNN algorithm was written to predict the data with the above data. Then to evaluate the program, a non-anticancer peptide, Temporin-Las peptide (Anticancer peptide) was subjected for the prediction of anticancer activity. The results were produced as graphs to visualize the prediction, which is shown in Figure 1.

In this figure 1, the fuchsia-colored lines indicate the referral anticancer peptide, and thegreen dot, indicates the sequence submitted to the program which extracts the features, reduces the dimension of thefeatures, and predicts the activity. The X-axis and Y-axisnumerals present in the graph don't show the prediction score. The prediction is determined only when the green dot touches any of the pink lines shows the anticancer activity and if the green dot doesn't touch the pink line, it means the sequence needs to be predicted doesn'thave any anticancer. From this program, GPGPG – linker, which is predicted as non-ACP, and Temporin-Las was predicted as the ACP.

Table 3: Shows the anticancer and cell-penetrating activity prediction of Temporin-Las and predicted sequence with SVM, RF, and XGBoost algorithms.

Temporin-Las (LLRHVVKILSKYL)		**Predicted Sequence (LAKNVVKILSKYL)**	
SVM_ACP	ACP	SVM_ACP	ACP
SVM_CPP	CPP	SVM_CPP	CPP
RF_ACP	ACP	RF_ACP	ACP
RF_CPP	CPP	RF_CPP	CPP
XGB_ACP	ACP	XGB_ACP	ACP
XGB_CPP	CPP	XGB_CPP	CPP

Figure 1: Shows the results for testing the KNN algorithm with the non-anticancer peptide (GPGPG – a peptide linker) and Temporin-Las (an anticancer peptide).

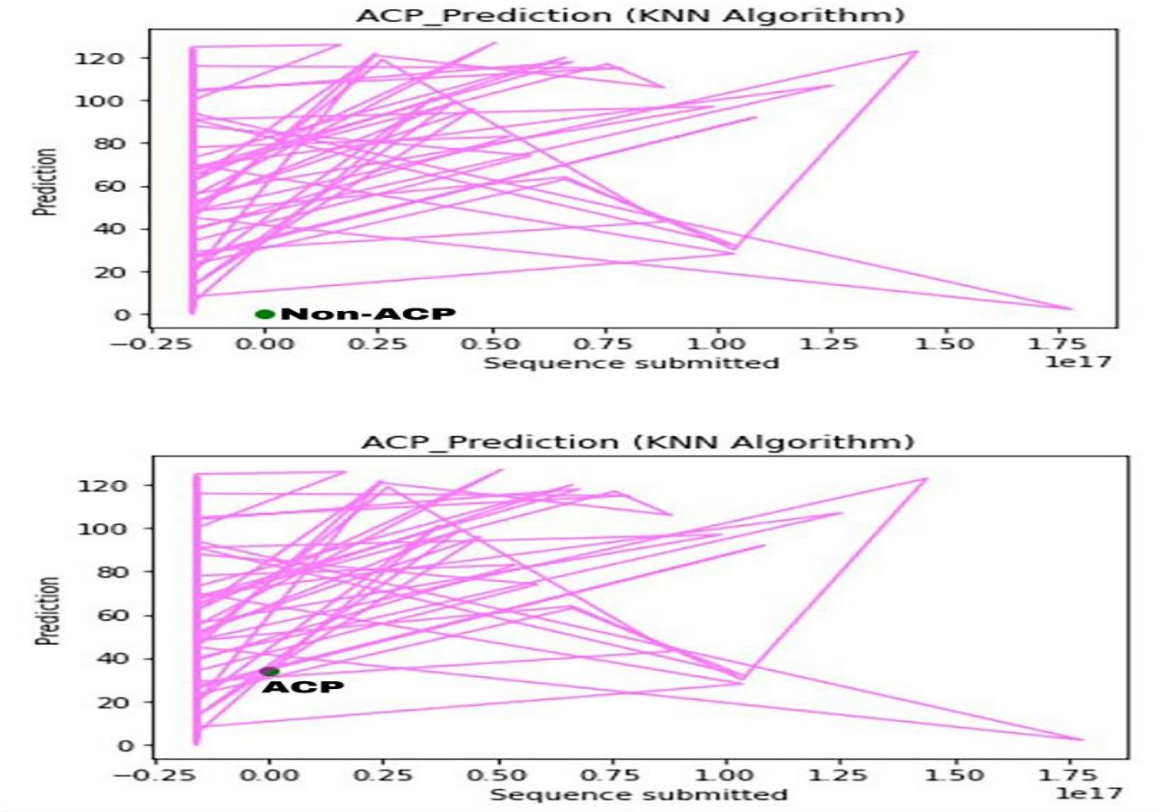


Table 5: Shows the mutated peptides which have anticancer activity in the *in-silico* model with SVM, RF, and XG Boost algorithms. It consists of 115 mutated peptides that have anticancer andcell-penetrating activity with all three algorithms.

AAKNVVKILSKYL	LMKNVVKILSKYL	LAKPVVKILSKYL	LAKNVYKILSKYL
CAKNVVKILSKYL	LNKNVVKILSKYL	LAKQVVKILSKYL	LAKNVVRILSKYL
IAKNVVKILSKYL	LPKNVVKILSKYL	LAKRVVKILSKYL	LAKNVVKHLSKYL
KAKNVVKILSKYL	LQKNVVKILSKYL	LAKSVVKILSKYL	LAKNVVKKLSKYL
MAKNVVKILSKYL	LRKNVVKILSKYL	LAKTVVKILSKYL	LAKNVVKLLSKYL
NAKNVVKILSKYL	LSKNVVKILSKYL	LAKVVVKILSKYL	LAKNVVKMLSKYL
PAKNVVKILSKYL	LTKNVVKILSKYL	LAKWVVKILSKYL	LAKNVVKRLSKYL
QAKNVVKILSKYL	LVKNVVKILSKYL	LAKYVVKILSKYL	LAKNVVKVLSKYL
RAKNVVKILSKYL	LWKNVVKILSKYL	LKNIVKILSKYL	LAKNVVKWLSKYL
SAKNVVKILSKYL	LYKNVVKILSKYL	LAKNLVKILSKYL	LAKNVVKIHSKYL
TAKNVVKILSKYL	LARNVVKILSKYL	LAKNWVKILSKYL	LAKNVVKIISKYL
VAKNVVKILSKYL	LAKAVVKILSKYL	LAKNVCKILSKYL	LAKNVVKIKSKYL
WAKNVVKILSKYL	LAKCVVKILSKYL	LAKNVFKILSKYL	LAKNVVKIMSKYL
YAKNVVKILSKYL	LAKFVVKILSKYL	LAKNVHKILSKYL	LAKNVVKIRSKYL
LCKNVVKILSKYL	LAKGVVKILSKYL	LAKNVIKILSKYL	LAKNVVKIVSKYL
LFKNVVKILSKYL	LAKHVVKILSKYL	LAKNVKKILSKYL	LAKNVVKIWSKYL
LHKNVVKILSKYL	LAKIVVKILSKYL	LAKNVLKILSKYL	LAKNVVKIYSKYL
LIKNVVKILSKYL	LAKKVVKILSKYL	LAKNVPKILSKYL	LAKNVVKILAKYL
LKKNVVKILSKYL	LAKLVVKILSKYL	LAKNVRKILSKYL	LAKNVVKILCKYL
LLKNVVKILSKYL	LAKMVVKILSKYL	LAKNVWKILSKYL	LAKNVVKILFKYL
LAKNVVKILGKYL	LAKNVVKILRKYL	LAKNVVKILSKIL	LAKNVVKILSKYM
LAKNVVKILHKYL	LAKNVVKILTKYL	LAKNVVKILSKKL	LAKNVVKILSKYN
LAKNVVKILIKYL	LAKNVVKILVKYL	LAKNVVKILSKLL	LAKNVVKILSKYQ
LAKNVVKILKKYL	LAKNVVKILWKYL	LAKNVVKILSKML	LAKNVVKILSKYR
LAKNVVKILLKYL	LAKNVVKILYKYL	LAKNVVKILSKRL	LAKNVVKILSKYS
LAKNVVKILMKYL	LAKNVVKILSRYL	LAKNVVKILSKVL	LAKNVVKILSKYV
LAKNVVKILNKYL	LAKNVVKILSKAL	LAKNVVKILSKWL	LAKNVVKILSKYW
LAKNVVKILPKYL	LAKNVVKILSKFL	LAKNVVKILSKYI	LAKNVVKILSKYY
LAKNVVKILQKYL	LAKNVVKILSKHL	LAKNVVKILSKYK	

Manual mutation of the predicted ACP sequence.

From the predicted ACP, the amino acid in each position of the sequence was changed without altering the length of the sequence to identify more anticancer sequences which can be more potent than the predicted sequence and create a database for the researchers to find the activity of the peptides *in vivo* and *in vitro*. The manually mutated 248 peptides were produced. These were considered as the mutated predicted lineage which is subjected to the CpACpP online tool to find their anticancer and cell-penetrating activity with all the three algorithms – SVM, RF, and XGBoost. Nearly 115 mutated peptides were identified, which are mentioned in Table 5.

Scrutiny of the selected mutated peptide lineage and analysis of predicted peptide sequence.

The selected 115 mutated peptides were scrutinized for the allergenicity property in the AllerTop v2.0 webserver. 84 predicted anticancer peptides were obtained from the AllerTop v2.0 web server as the non-allergic peptides. Then these non-allergic peptides were checked for their stability with the assist of the ProtParam webserver. 80 peptides were found to be highly stable, and 4 peptides were predicted as the unstable peptide. Considering the effect of stability in future formulations, the unstable peptides were omitted and only the 80 stable peptides were considered for the next study. It is also necessary to uncover the stability and allergenicity of the predicted sequence with the help of ProtParam and AllerTop v2.0 webserver. The results of the predicted sequence us given in Table 6 and the 80 stable, non-allergic anticancer peptides of predicted mutated lineage were mentioned in Table 7.

Table 6: Shows the stability and allergic properties of the predicted anticancer peptide.

Sequence	Stable / Unstable	Allergen / Non-Allergen
LAKNVVKILSKYL	Stable	Non-Allergen

Table 7: Shows the list of non-allergic, highly stable, an anticancer peptide from the mutantlineage of the predicted sequence which will be further studied for the anticancer activity againstthe A549 cell line.

AAKNVVKILSKYL	LAKNVVKILKKYL	LAKNVVKILSRYL	LAKNVVRILSKYL
IAKNVVKILSKYL	LAKNVVKILLKYL	LAKNVVKILSKFL	LAKNVVKHLSKYL
KAKNVVKILSKYL	LAKNVVKILMKYL	LAKPVVKILSKYL	LAKNVVKKLSKYL
MAKNVVKILSKYL	LAKNVVKILNKYL	LAKVVVKILSKYL	LAKNVVKLLSKYL
NAKNVVKILSKYL	LAKNVVKILPKYL	LAKWVVKILSKYL	LAKNVVKMLSKYL
PAKNVVKILSKYL	LPKNVVKILSKYL	LAKYVVKILSKYL	LAKNVVKRLSKYL
QAKNVVKILSKYL	LRKNVVKILSKYL	LKNIVKILSKYL	LAKNVVKVLSKYL
SAKNVVKILSKYL	LWKNVVKILSKYL	LAKNWVKILSKYL	LAKNVVKWLSKYL
TAKNVVKILSKYL	LARNVVKILSKYL	LAKNVFKILSKYL	LAKNVVKIHSKYL
VAKNVVKILSKYL	LAKAVVKILSKYL	LAKNVHKILSKYL	LAKNVVKIMSKYL
WAKNVVKILSKYL	LAKFVVKILSKYL	LAKNVIKILSKYL	LAKNVVKIRSKYL
YAKNVVKILSKYL	LAKGVVKILSKYL	LAKNVKKILSKYL	LAKNVVKIYSKYL
LFKNVVKILSKYL	LAKHVVKILSKYL	LAKNVLKILSKYL	LAKNVVKILCKYL
LHKNVVKILSKYL	LAKIVVKILSKYL	LAKNVRKILSKYL	LAKNVVKILFKYL
LAKNVVKILAKYL	LAKLVVKILSKYL	LAKNVWKILSKYL	LAKNVVKILSKYN
LKKNVVKILSKYL	LAKMVVKILSKYL	LAKNVVKILSKYM	LAKNVVKILSKYR
LLKNVVKILSKYL	LAKNVVKILTKYL	LAKNVVKILSKRL	LAKNVVKILSKYS
LAKNVVKILGKYL	LAKNVVKILSKYQ	LAKNVVKILSKYI	LAKNVVKILSKYV
LAKNVVKILHKYL	LAKNVVKILWKYL	LAKNVVKILSKYK	LAKNVVKILSKYW
LAKNVVKILIKYL	LAKNVVKILYKYL	LAKNVYKILSKYL	LAKNVVKILSKYY

Anticancer activity range prediction against lung cancer cell line – A549

The Temporin-Las predicted anticancer sequence and 80 anticancer peptides from the mutated lineage of the predicted sequence were subjected to predict the intensity of the anticancer activity with xDeep-AcPEP. The results were obtained for the Temporin-Las, predicted anticancer sequence, and 80 anticancer peptides from the mutated lineage of the predicted sequence. One best peptide sequence was selected from the 80 anticancer peptides (Mutant lineage) with the results of xDeep-AcPEP and named as SSVAM-9 (LAKNVVKILSKYS) in Table 8. The sequence which is predicted with the help Temporin-Las is named SSVAP-3. The results of Temporin-Las, SSVAP-3, and SSVAM-9 predicted with xDeep-AcPEP were compared in Table 9. It was also confirmed by the KNN algorithm and visualized as graphs in Figure 2.

Table 8: Shows the prediction of 80 anticancer peptides (Mutant lineage) with the xDeep-AcPEPprogram and the highest prediction scored peptide was chosen as SSVAM-9.

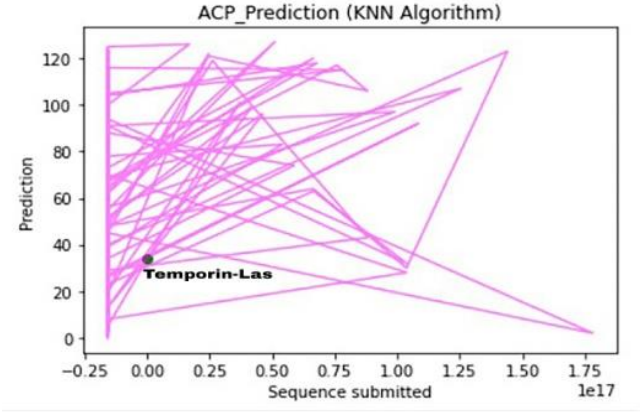
Mutant sequence	Prediction against lung carcinoma	Mutant sequence	Prediction against lung carcinoma
AAKNVVKILSKYL	212.4759	LAKNVVKILWKYL	24.17543
IAKNVVKILSKYL	130.99	LAKNVVKILYKYL	46.53145
KAKNVVKILSKYL	187.1467	LAKNVVKILSRYL	110.3005
MAKNVVKILSKYL	114.3553	LAKNVVKILSKFL	64.70927
NAKNVVKILSKYL	145.1239	LAKPVVKILSKYL	105.199
PAKNVVKILSKYL	142.7539	LAKVVVKILSKYL	97.08617
QAKNVVKILSKYL	159.1972	LAKWVVKILSKYL	38.7039
SAKNVVKILSKYL	164.7539	LAKYVVKILSKYL	77.06455
TAKNVVKILSKYL	164.2149	LKNIVKILSKYL	81.51817
VAKNVVKILSKYL	143.4366	LAKNWVKILSKYL	39.40437

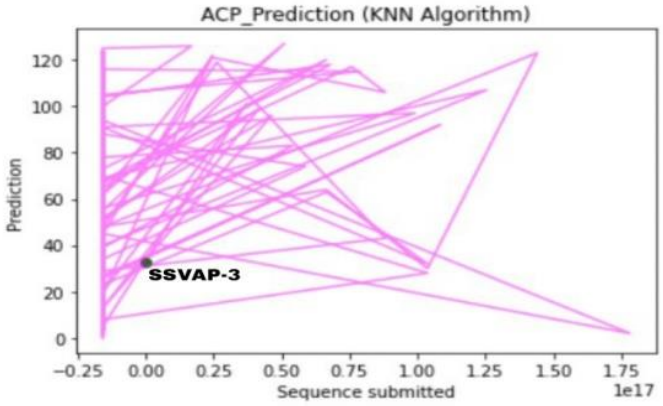
WAKNVVKILSKYL	62.77576	LAKNVFKILSKYL	62.13508
YAKNVVKILSKYL	119.7523	LAKNVHKILSKYL	132.9407
LFKNVVKILSKYL	41.19028	LAKNVIKILSKYL	89.86398
LHKNVVKILSKYL	65.88494	LAKNVKKILSKYL	146.7037
LAKNVVKILAKYL	116.8738	LAKNVLKILSKYL	59.11219
LKKNVVKILSKYL	86.68785	LAKNVRKILSKYL	147.2667
LLKNVVKILSKYL	44.10253	LAKNVWKILSKYL	39.98738
LAKNVVKILGKYL	108.6707	LAKNVVKILSKYM	181.1988
LAKNVVKILHKYL	83.75097	LAKNVVKILSKRL	190.0145
LAKNVVKILIKYL	42.2131	LAKNVVKILSKYI	159.5082
LAKNVVKILKKYL	79.45282	LAKNVVKILSKYK	320.7467
LAKNVVKILLKYL	27.64566	LAKNVYKILSKYL	86.50407
LAKNVVKILMKYL	42.49052	LAKNVVRILSKYL	100.8169
LAKNVVKILNKYL	89.13308	LAKNVVKHLSKYL	168.8379
LAKNVVKILPKYL	92.56837	LAKNVVKKLSKYL	168.3865
LPKNVVKILSKYL	64.55414	LAKNVVKLLSKYL	64.11542
LRKNVVKILSKYL	75.59894	LAKNVVKMLSKYL	96.57786
LWKNVVKILSKYL	29.44689	LAKNVVKRLSKYL	179.2148
LARNVVKILSKYL	89.18212	LAKNVVKVLSKYL	112.4134
LAKAVVKILSKYL	164.8712	LAKNVVKWLSKYL	47.98902
LAKFVVKILSKYL	58.69478	LAKNVVKIHSKYL	284.8632
LAKGVVKILSKYL	113.1316	LAKNVVKIMSKYL	166.4956
LAKHVVKILSKYL	106.6791	LAKNVVKIRSKYL	297.4304
LAKIVVKILSKYL	83.54928	LAKNVVKIYSKYL	179.3604
LAKLVVKILSKYL	59.6705	LAKNVVKILCKYL	39.81289
LAKMVVKILSKYL	72.37469	LAKNVVKILFKYL	31.29918
LAKNVVKILTKYL	85.81689	LAKNVVKILSKYN	373.3158
LAKNVVKILSKYQ	341.6746	LAKNVVKILSKYR	347.8812
LAKNVVKILSKYS	**398.9989**	LAKNVVKILSKYW	100.3618
LAKNVVKILSKYV	167.0566	LAKNVVKILSKYY	203.7023

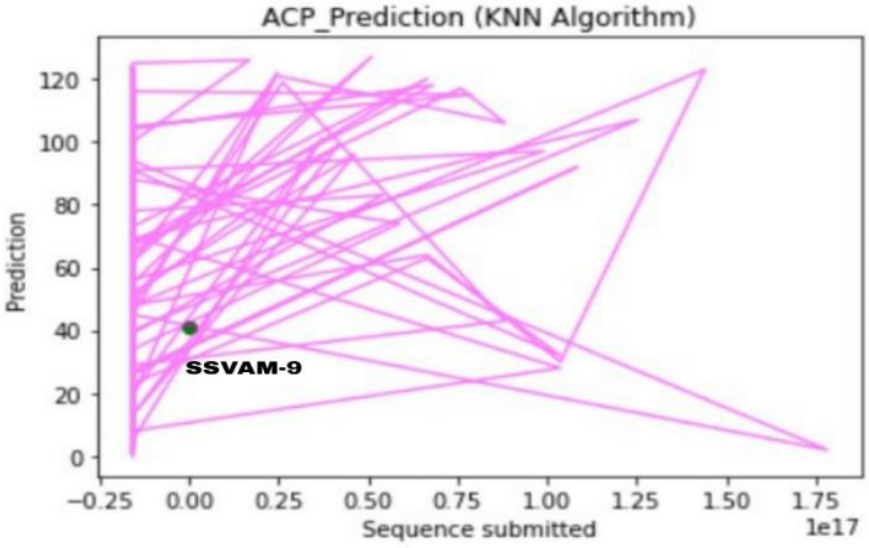
Table 9: Shows the prediction results of the anticancer peptides against the A549 cell line withthe xDeep-AcPEP program.

Peptides	Activity prediction against lung carcinoma
Temporin-Las (LLRHVVKILSKYL)	44.72525
SSVAP-3 (LAKNVVKILSKYL)	101.9028
SSVAM-9 (LAKNVVKILSKYS)	398.9989

Figure 2: Shows the prediction of the Temporin-Las, SSVAP-3, and SSVAM-9 with the KNNalgorithm (Own program) which shows the activity of each in the graph. The peptide SSVAM-9 has higher activity predicted by the AcPEP program and our program which uses the KNN algorithm. Even in the graph, the SSVAM-9 had placed between the intense lines compared to other peptides,confirming the excellent anticancer activity of SSVAM-9.







CONCLUSION

Thus, anticancer peptides are an alternative way of treatment against cancer [13]. In this study, we have nearly evaluated all the mutations of a particular predicted sequence. This also creates a database for the stable,non-allergic anticancer peptides which support the researchers and scientists to research about the anticancer peptides and develop new novel ACPs. With the above-predicted

results, we had isolated one best peptide (SSVAM-9) from 80 mutated peptides and was compared it with the predicted sequence (SSVAP-3). From the predictive results of Temporin-Las, SSVAP-3, and SSVAM-9, we conclude that SSVAM-9 is a novel anticancer peptide against the lung carcinoma. It is stable, non-allergen with high potential in this*insilico* model. SSVAM-9 can be easily formulated, as this peptide is more stable than other ACPs predicted in this study. This is a preliminary study that can be further evaluated in *invivo* and *in-vitro* models.

Conflict of interest:
All authors confirm there is NO conflict of Interest.

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