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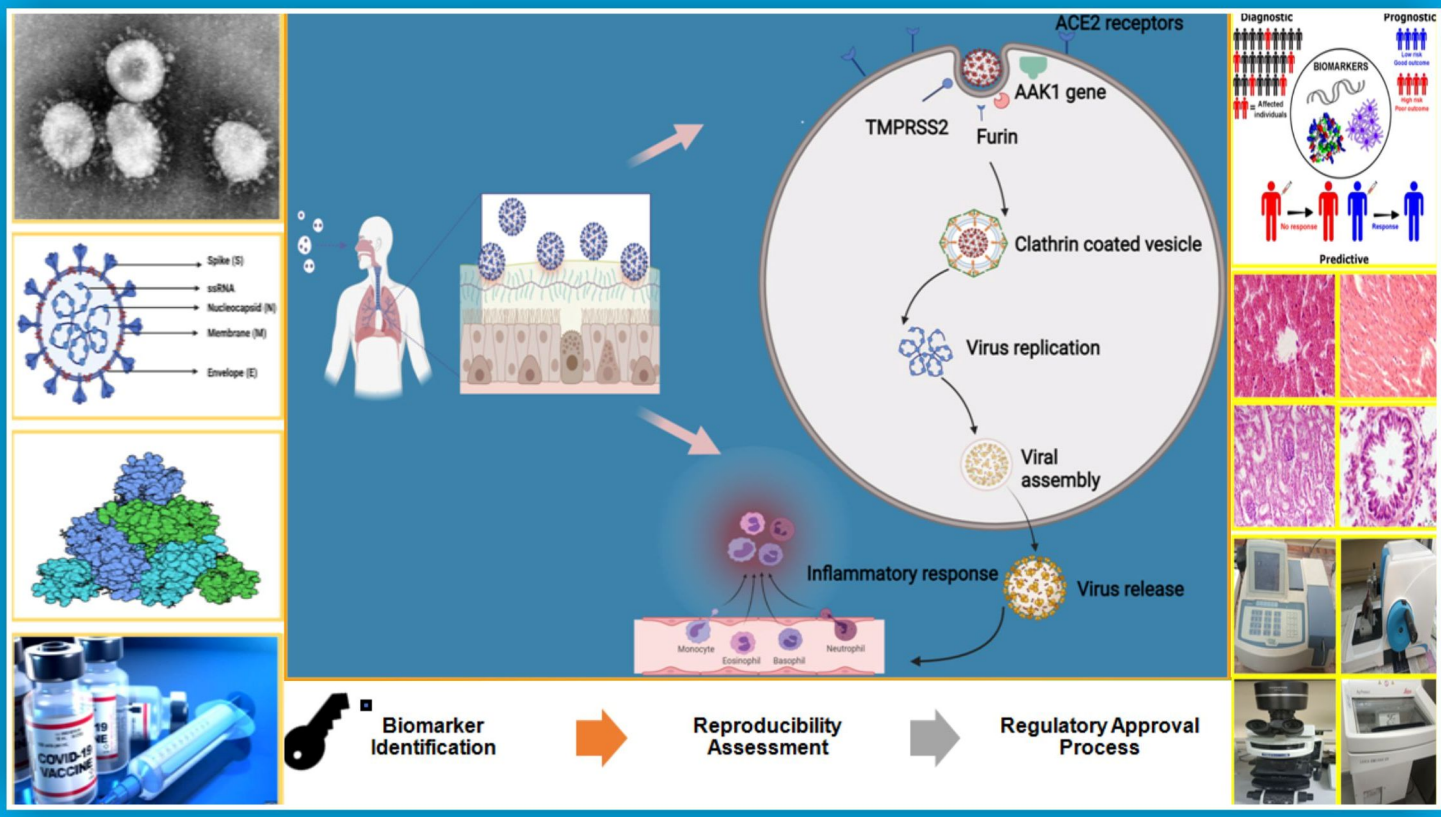


## Azadi Ka Amrit Mahotsav

## Artificial Intelligence in Drug Discovery(AIDD)

## Biomarkers in COVID-19: Prospects and Problems

## The six challenges to pharmacy practice in India

## CRIPS Digest & NIPER News

National Institute of Pharmaceutical Education and Research (NIPER)
S.A.S. Nagar

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## EDITORIAL

It's a pleasant occasion to note CRIPS publication has been revived. Due to some unavoidable circumstances, there was a gap of 3 years in the publication. As our institution is being instilled with new energy and stability, it has become practical to revive this publication. I am confident that the readers will appreciate this new enthusiasm as well as subscribe and support the upcoming CRIPS issues as usual.

In this issue, the importance of Artificial Intelligence in Drug Discovery (AIDD) has been highlighted. This emerging technology has a lot of promise though it is in its infancy. Several techniques under this umbrella are being explored towards the drug design. Machine learning (ML) methods like Support Vector Machine (SVM), Artificial Neural Network (ANN), Deep Learning (DL), Genetic Algorithm (GA), Knowledge Base System (KBS), etc. are being efficiently integrated along with stabilities to design new drugs. These methods are significantly different from the quantum medicinal chemistry and molecular mechanics methods, which deal with electrons and atoms in the drugs respectively. The AIDD methods are strongly associated with data analytics. In this issue, the current status and the future prospects of AIDD have been covered.

COVID-19 pandemic has prompted significant drift the drug discovery research. In India mostly virus biology and virus epidemiology related topics were extensively studied, but less importance was given for the antiviral drug discovery. Since past 2 years due to COVID-19 pandemic, a lot of research activities in vaccine development as well as drug development have been taken-up. To identify the COVID-19 virus induced disease conditions several biomarkers are required for proper diagnosis and treatment specifically for the comorbidities. The prospects and problems associated with biomarker development in COVID-19 have been discussed in the current issue.

Pharmacy practice topic is essential to bridge between pharmacist, physician and the patients. Though its importance was realized since long time, there were several practical challenges in adopting this technology in India. Prof. Tiwari elaborated six different challenges being experienced by pharmacy practice experts in India.

**P.V. Bharatam**




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# Azadi Ka Amrit Mahotsav

India is celebrating Azadi Ka Amrit Mahotsav (AKAM). Many events in the field of pharmaceutical sciences were held under the aegis of Department of Pharmaceuticals, Ministry of Chemicals and Fertilizers. NIPER SAS Nagar organized "NIPER Week" from 4th-9th October, 2021" as a common program for all seven NIPERs. These events were conducted in both offline and online modes at NIPER, SAS Nagar for one week.

Many pharmaceutical industry experts, academicians, start-up leaders, and NIPER alumni gave lectures during this week. Dr. Mansukh Mandaviya, the Hon'ble Union Minister (Ministry of Chemicals and Fertilizers), was the Chief Guest and Ms. S. Aparna, Secretary, Department of Pharmaceuticals, Chairperson, Apex Council, NIPERs was the Guest of Honour for the inaugural event. There were inaugural lectures on the theme "Story of Pharma sector during last 75 years". The lectures were by Dr. Rustom Mody, Head Biologicals, Sun Pharma, Dr. Dinesh Dua, Executive Director, Chairman, Nectar Lifesciences, & Chairman, Pharmexcil and Chairman, CII Northern Region, (Life Science and Biotech) and Dr. Girish Sahni, Ex DG-CSIR. There was a key note address by Dr. V.K. Paul, Member, NITI Aayog.

During Industrial Leadership Conclave, four speakers talked on the theme "The role of Pharmaceutical industry in development of India since Independence". The speakers were Dr. Ved Shrivastava, Vice President of Chemistry at Aktis Oncology, NC, USA, Dr. Rajamannar Thennati from Sun Pharma Advanced Research Co. Ltd. and SPARC Bio-Research Pvt Ltd., Dr. Gautam Das, Co-Founder and Managing Director for Startup miBiome Therapeutics, Mumbai and Dr. Sanjay Singh from Gennova Biopharmaceuticals, Pune.

On October 6th 2021, under the category of Popular Science Lectures (Academia), four eminent speakers gave talks on the theme "Drug Discovery @ 75". The session started with the talk of Padma Bhushan awardee Prof. P. Balaram, Ex-Director, IISc, Bengaluru. The session continued with a talk by Dr. Shekhar C. Mande, Director General, DSIR-CSIR, Prof. Mitali Chatterjee, Prof. & Head, Department of Pharmacology, Institute of PG Medical Education and Research, Kolkata and Prof. Javed Iqbal, Ex-Director, Dr Reddy's Institute of Life Sciences, Hyderabad.

On the next day, six NIPER Alumni gave presentations on the theme "Experience at NIPER and in the real world". During the AKAM Celebrations, two exhibitions were organized in the institute on (i) History of Pharmacy in India and (ii) Medicinal Plants.

Under the heading "Popular Science Lectures", the lectures were delivered by the BoG Members / Superannuated faculty members of NIPER on the theme "Pharmaceutical Education and Research @ 75". Dr. Mukul Jain, President, Zydus Research Centre, Ahmedabad, BoG - NIPER Ahmedabad, Prof. Asit K. Chakraborti, Ex-Professor & HoD, NIPER SAS Nagar, Prof. U.C. Banerjee, Ex-Professor & HoD, NIPER SAS Nagar and Prof. P.P. Singh, Ex-Professor, NIPER SAS Nagar delivered their talks.

On 11.10.2021, a one day seminar was conducted by Shin-Etsu in collaboration with AAPS NIPER Student Chapter on the title 'Pharmaceutical Excipients for Solid Dispersion". This seminar focused on providing critical inputs on excipients available with "Shin-Etsu" for preparing solid dispersions.

From 18.10.2021, three lectures were conducted by Agilent and AAPS NIPER Student Chapter. The first lecture was on the title 'Dissolution testing solutions for emerging formulations: Definitive Nanoparticle Dissolution testing". The lecture imparted critical insights regarding NanoDis system by Agilent. On 20.10.2021, a second lecture on the title "Dissolution testing solutions for emerging formulations: Biorelevant Dissolution testing" was delivered. The lecture explained the technology development background for USP type III apparatus by Agilent. Further, Technology overview of BioDis Apparatus III and Reference applications of the same were elaborately discussed. On 22.10.2021, a third lecture on the title "Dissolution testing solutions for emerging formulations: Compendial small volume Dissolution testing for novel dosage forms such as combination drug products" was delivered. The lecture focused on 400 DS Apparatus VII launched by Agilent. This allowed attendees to gather critical inputs for their dissolution studies.

Prof. Dulal Panda the Director of the institute distributed the prizes to the winners of the Poster making and Essay Writing Competitions held on 26th and 27th October, 2021. He felicitated Retd. Air Force Officer, Sqn. Ldr. Chandra Prakash for his remarkable contributions to the nation.

On 31.10.2021, there were two lectures organized. First entitled "Post-independence Developments in Indian Pharmaceutical Industry Some Toxic Dependence and What Country is Doing to Overcome It" By Prof. HPS Chawla, Ex-Dean, NIPER, SAS Nagar and second entitled "The Idea of Bharat: A Rashtra or Nation?" by Prof. Sudhir Kumar, Professor, Panjab University, Chandigarh. There was Sitar Vadan by Pt. Shubhendra Rao under the aegis of SPIC MACAY.

Current Research & Information on Pharmaceutical Sciences (CRIPS)
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Review Article

# Artificial Intelligence in Drug Discovery (AIDD)

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The importance of Computer Aided Drug Discovery (CADD) has been consistently increasing over the past 20 years. Artificial Intelligence (AI) is one of the CADD methods, machine learning (ML) is one of its subtopics. In the past five years, many Artificial Intelligence in Drug Discovery (AIDD) approaches were employed towards drug discovery. This article includes discussion on the scope and limitations of AIDD.

## Introduction

The Artificial intelligence (AI) "is the science and engineering of making intelligent machines, especially intelligent computer programs. It is related to the similar task of using computers to understand human intelligence, but AI does not have to confine itself to methods that are biologically observable".[1,2] In the year 1956, Allen Newell and his colleagues created the Logic Theorist, the first running AI software program.[3] Though the original objectives of AI are not yet completely realized, many AI techniques became available and their usage is increasing with time. The AI techniques are very popular now-a-days due to the involvement of AI in its various forms across a large range of domains ranging from robotics, speech translation, image analysis, etc. Several innovative techniques were developed by computer science researchers in an attempt to make computers intelligent, some of them found applicable in chemistry, biology and pharmaceutical sciences. To design new organic synthetic schemes, to understand complex biological systems, to design new APIs or development of new analytical/ diagnostic devices or methods, AI is being used. The AI techniques are also applicable to drug discovery, drug development, drug repurposing, drug metabolism prediction, drug toxicity analysis, improving pharmaceutical productivity, clinical trials and almost all aspects of pharmaceutical sciences.[4] All these techniques are collectively considered under AIDD (Artificial Intelligence in Drug Discovery). These AI technologies are not yet routinely practical in Computer Aided Drug Design (CADD), yet they are being used to resolve complex drug discovery problems. In comparison to Ligand-based drug design (LBDD) and Structure-based drug design (SBDD), AIDD is in its nascent state. A few books are available which include discussion on AIDD.[5,6]

Initially, AI was utilized to develop logical programming platforms (Prolog,[7] LISP[8]) on par with general programing languages. Later on, as a part of machine learning (ML),[5, 9,10] many novel methods Knowledge Base Systems (KBS) like Artificial Neural Networks (ANN), Support Vector Machines (SVM), Genetic Algorithms (GA), Deep Learning (DL) Fuzzy Systems (FS), pattern recognition tools, classifiers, etc. were introduced, all of them found applications in AIDD.[11,12] In the current scenario, the AI methods are emerging complementary to the molecular modelling based methods for the CADD scientists. As of today, it appears that the AIDD based operations are different from those operations offered at atomic level by molecular modelling techniques, however, the overlap between these operations are increasing with an exponential growth.

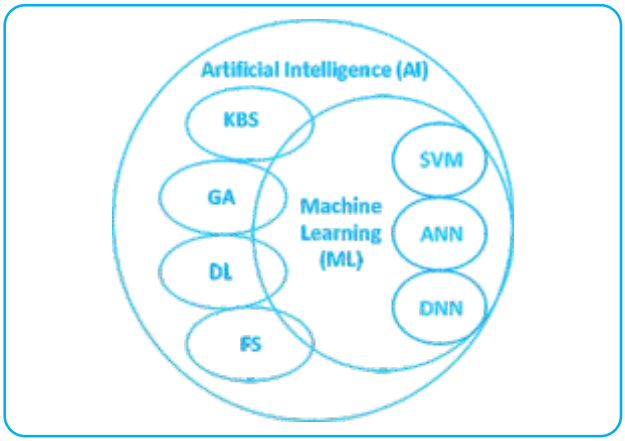


**Figure 1:** Artificial Intelligence in Drug Discovery (AIDD)

As the contributions of ML in Drug Discovery (MLDD)[5,9, 10,13] are increasing, the terms AIDD and MLDD are being treated as synonyms. Machine learning and data mining can be applied to provide many solutions such as -- classification, regression, clustering, dimensionality reduction, reinforcement learning, deep learning, anomaly detection and many more. Apart from that, several subtopics of machine learning include ANN, DNN (Deep neural networks), RNN (Recurrent neural networks), CNN (Convolutional neural networks), GA, SVM, Bayesian Networks, DT (Decision trees), LR (Logistic regression), k-NN (k-nearest neighbors), NB (Navie bayesian) techniques are also important in CADD (Figure 1).

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## Knowledge-Based Systems (KBS) in Drug Discovery

A knowledge-based system[14] (KBS) is a computational approach that captures and uses knowledge from a variety of sources. Knowledge Base (KB) is a data base of knowledge, Information related to any particular topic is stored in the form of Fact Bases (FB) and the relations between various factual data points in Fact Bases are defined in the formof Rule Bases (RB). When the data from FB and RB are efficiently interpreted with the help of inference engines, the entire system is known as an expert system (ES). Currently, a knowledge-based system is a major area of artificial intelligence which can help in making decisions based on the data and information that resides in their database i.e. Knowledge Base (KB). Several Drug-KBS are being developed.[15] PharmGKB from Stanford University is a KBS based on drug-gene interactions,[16] DailyMed is a KBS containing drug-disease information,[17] SuperTarget is a KBS which includes information related to drug-target interactions,[18] merged-PDDI includes drug-drug interaction KBS.[19] These drug-KBS are being used for drug repositioning by identifying new drug indications with the help of knowledge on drug-target, protein-protein, gene-disease interactions. Beneficial drug combination predictions are being made from the drug-target information after combining with ATC (Anatomical Therapeutic Chemical) classification systems. Though there are a few successes, many issues are yet to be sorted out and the applications are yet to be expanded, for example, in terms of drug-KBS integration, implementation, improving predictive results, eliminating negative samples, etc.[20]

In the field of CADD, the calculation of several descriptors for QSAR purpose is based on knowledge-based system. Knowledge-based scoring functions (for molecular docking purposes) are application of KBS which rely on the statistical observations of intermolecular contacts collected in large 3D structural databases. Chemical and macromolecular databases (CCSD and PDB) stored potential mean forces (distance dependent) for various subunits of molecules, because many such interactions occur frequently among the small and macromolecules. SmoG, ASP, DSX, IT-Score, DrugScore, etc. are some of the known scoring functions which are based on the KBS methods.[21] For example, DrugScore scoring function which employs the distance-dependent pair potentials from nonbonded interactions, has been derived from the crystal data. The scoring functions $DrugScore^{CSD}$ and $DrugScore^{PDB}$ are derived from the crystal data from CSD and PDB respectively.[22] Klebe and his co-workers established that $DrugScore^{CSD}$ provides relatively more satisfactory results compared to the original PDB-based DrugScore.[22] Huang and co-workers developed a distance-dependent knowledge-based scoring function - ITScore-PP to predict protein-protein interactions. They utilized crystal structures of 851 dimeric protein complexes which containing true biological interfaces to derive ITScore-PP scoring function.[23] Ebejer et al. developed Ligity: a non-superpositional, knowledge-based method to perform virtual screening of small molecules.[24] Several ADME-Tox property based quantitative values are also being estimated using knowledge based methods.[25]

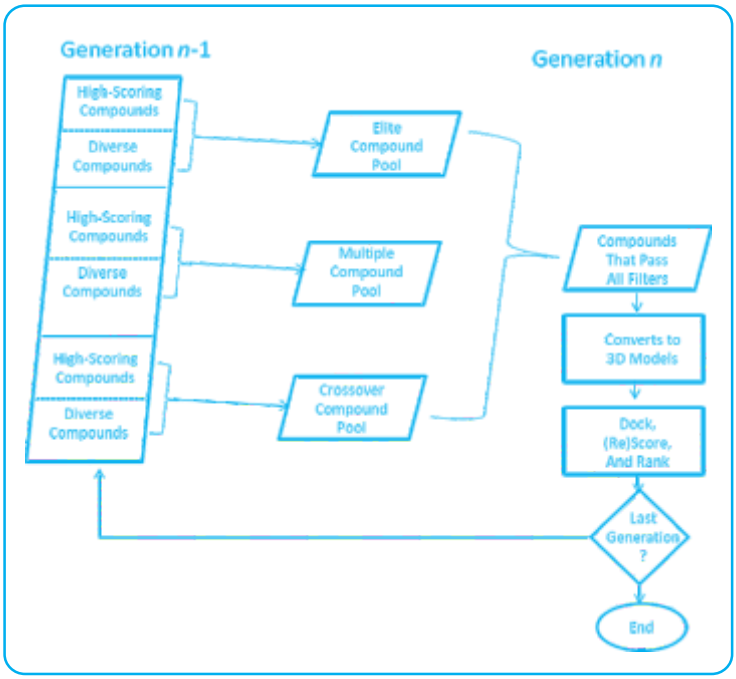


**Figure 2**: Process-flow diagram of the AutoGrow4 algorithm. Figure adopted from Ref.[30]

## Genetic Algorithms (GA) in Drug Discovery

The genetic algorithms (GA)[26] is a computational method for solving both constrained and unconstrained optimization problems by adopting natural selection procedure. GAs "evolve" solutions to problems using the principles of genetics. Several generations of solutions are considered which involve many candidate solutions in each generation. The transformation of data from one generation to the next generation happens mainly in three ways - (i) as per a fitness function (ii) by crossover process and (iii) mutated in a systematic manner. In this way, the number of candidate solutions gets reduced marginally and the solutions in the next iteration get better. When this iterative procedure is continued for a few generations, fittest solutions are obtained.[26,27]

GAs are being employed in QSAR and molecular Docking extensively. Genetic function approximation can be used as an alternative to regression analysis.[28] They are being used for descriptor selection, by performing generations of QSAR analysis. A combination of QSAR and GA methods were employed for designing inhibitors of methyl transferase by Sun et al.[29] Additional MLR analysis was employed to obtain statistical significance. The identified descriptors are ionization potential, topological charge indices, polarizaility, and number of aromatic amines in a molecule.[29]

Many molecular docking algorithms are based on GA approach. Initially many conformers are considered in the first generation, as the generations progress, the number of conformers gets reduced-based on the best fit docked

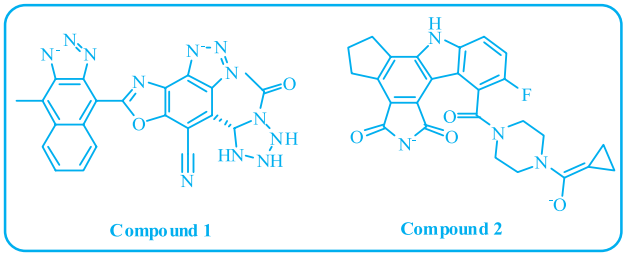


**Figure 3:** Compounds finalized from AutoGrow4 analysis.[30]

conformers. Each conformer is scored based on knowledge - based energy function. The conformers present In the final generation are subjected to validation using other CADD and experimental methods.[31] Autodock (Lamarkian Genetic Algorithm) and Glide software (Exhaustive search algorithms) utilizes GA based molecular docking approach.

Auto Grow4 is an open-source GA based lead optimization software.[30] It is based on python coding, it is useful for predicting novel ligands using a combination of GA and molecular docking. A population of seed molecules is chosen initially as potential solution. Molecular docking of these ligands into a chosen targets helps in identifying suitable molecules towards the next generation. The propagation of ligands from one generation to another generation can be done using crossover, mutant compound generation, elite compound selection, filtration, assessing the fitness, evaluating chemical properties, etc. All these help in lead optimization (Figure 2). Spiegel and Durrant demonstrated the use of AutoGrow4 by designing PARP-1 inhibitors. They identified two compounds from this analysis (Figure 3). MoleGear is also a useful evolutionary approach to *de novo* design.[32] GANDI utilizes GA approach in parallel mode for lead optimization.[33]

## Machine learning (ML) in Drug Discovery

Machine learning (ML)[9,10,13,34-36] approaches provide a set of tools that can improve discovery and decision making for well-defined questions with abundant high-quality data. Computer software can be trained to grasp the important information related to drugs and allow the identification from millions of chemical species. Machine learning (ML) is a computer aided technique in which a set of data is provided to train some software component or to generate ML models which infer patterns from the supplied data to make reasonable predictions on the new data. In ML, hardware component does not learn anything from the supplied data. ANN, SVM, RF, LR, NB, etc. are the most successful ML methods which are being employed in drug discovery.[35,36] In this article, only DNN based drug discovery examples are included for brevity.

DNN is one of the latest developments in the field of ML, which is being used in CADD.[37-41] DNN contains multiple hidden layers (Figure 4). In DNN, nonlinear relations between the input parameters and the output can be effectively grasped. Mostly they are trained using the feedforward approach. In DNN, the neurons multiply the inputs and weights and take a decision to give an output signal in binary form (0 or 1). The weights get adjusted during training. Over fitting is one of the major issues in DNN, which can be overcome with improved data quality. In comparison to other NN models, DNN can handle thousands of parameters in the input layer - pre-selection of descriptors is not essential. Input layer can utilize descriptors acquired from 2D/3D structures as well as from the molecular finger prints. By modulating the number of layers, number of nodes in each layer, the activation function and other characteristics, we can fine-tune the performance of a DNN. Several benchmark studies were reported, which established that DNN performs better than RF, SVM, etc. methods. Similarly, a few studies were performed establishing that the multi-task DNN performs better than sing-task DNN. DNN methods perform better with an increase in the sample size - i.e. the larger the number of chemotypes to train a DNN, the better is its applicability (this factor may also be considered as a limitation of DNN as the models with small datasets may not perform satisfactorily). Identifying new chemical structures carrying desirable molecular properties (clogP, drug-likeness, etc.) is being carried out using DNN. Big data analysis using AI techniques is an important aspect, which is being effectively carried out using DNN. In the past 10 years, several reviews were published which report the application of DNN in *de novo* drug design, suggesting synthetic routes, prediction of binding affinity, estimation of activity properties, evaluation of ADMET properties.[37,39-41]

DeepTox pipeline which predicts toxicity of drugs is one of the important tools using Deep Learning approach.[41] Before taking up model building, standardization of data was done. A data set of 12,707 compounds was initially reduced to 8694 fragments after normalization and merging in DeepTox. Model validation was done using cluster cross validation approach. Platt scaling approach was used for ensemblepredictions. The DNN model

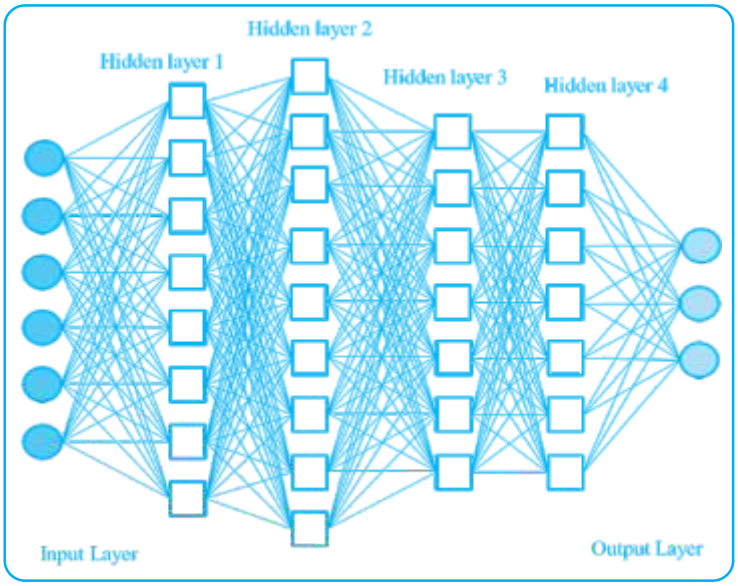


**Figure 4:** A representative diagram of Deep Neural Network. Figure adopted from Ref.[4]

outperformed the oth ermodels based on SVM, RF, etc.

Multi task DNN methods for classifying highly and weakly potent protein Kinase inhibitors[42] using DNN. A set of 19,030 potent inhibitors which possessed activity against 103 different human kinases were utilized for this study. This work is a multi-task DNN model. It was established that using DNN approach, the chemical features of kinase inhibitors can be effectively utilized for the classification purpose. The compounds exhibiting $pIC_{50}$ < 10nM were considered as highly potent and compounds exhibiting $pIC_{50}$ > 1000 nM were considered as weakly potent. Here, only those kinase targets were considered for the model development which possessed five positive as well as five negative inhibitors. The input data was based on finger prints (ex.ECFP4 based model contained 1127 bits). Out of the many generated DNN models, one model contained three hidden layers consisting of 2000, 1000 and 100 neurons. Back propagation algorithm was used to train the DNN. Over-fitting of the DNN was controlled using an algorithm (dropout - 25%) which was specifically known for this purpose. This model was shown to be better than many other alternatives considered for the classification of kinase inhibitors.[43]

AI techniques are generally considered as techniques which cannot explain the logic employed in drug discovery. However, a few attempts are being made to eliminate this limitation. Schneider and co-workers recently elaborated the current attempts in AIDD with the advantage of explainable artificial intelligence.[44] DNN is being used to model nonlinear relationships between input parameters of drug like molecules vs. the output parameters related to therapeutic application. DNN methods which are better than QSAR models are being developed for drug discovery. Chemception is a DNN tool developed for this purpose.[45, 46] Explainable artificial intelligence (XAI) is an approach for application in interpretable machine learning, such new approaches are bridging the gap between the traditional scientific approaches and the ML approaches. Transparency, justification, informativeness as well as uncertainty estimation are properties being offered by XAI.[47] The lack of liaisoning (if any) between the data scientists, chemoinformatics scientists, quantum medicinal chemistry experts, and synthetic medicinal chemistry experts is expected to be blurred as a result of XAI in drug discovery. Todeschini and co-workers adopted an integrated gradients feature attribution method in combination with a graph-CNN (convolutional neural network) to predict drug-cytochrome interaction. The site of metabolism (SoM) as well as the known metabolites could be predicted with the XAI approach.[48]

Methods for visually explaining the protein-ligand binding affinity, protein-ligand scoring as well as lead optimization of small molecule potency using three-dimensional CNN was introduced. KDEEP, DeltaDelta neural networks are the examples of 3D-CNN approach.[49,50] Molecular property prediction leading to *de novo* drug design is being suggested using graph-CNN approaches. Subgraph identification approaches (GNNExplainer) and attention-based approaches are also part of the graph-CNN efforts in chemistry/drug discovery.[51] Lipinski et al. elaborated the perspectives of deep learning applications in drug design and discovery.[52] Fooladi recently reviewed the existing RNN, CNN and DNN applications in drug design and discovery.[53]

## Limitation of AI in Drug Discovery

Although the efficacy of AI based methods in drug discovery are significant but their applications are limited in both capability and functionality.[54] One major criticism of many AI techniques such as neural networks is that they are often regarded as black boxes that merely attempt to map a relationship between output and input variables based on a training data set. This also immediately raises some concerns about the ability of the tool to generalize to situations that were not well characterized in the data set. One of the limitations of the genetic algorithm methods is that they are never guaranteed to reach the "optimal" solution, though the solutions provided are highly useful. In ML technique, we can not ensure that what the model learned in terms of derivitization or in terms of heuristic reasoning, the ML model itself learns a few factors from the data provided to it. It is difficult to ensure which factor of the supplied data was utilized to train which component of an ML model. A well-known drawback of deep learning is its poor performance where data size is low-to-medium.

## Conclusions and future perspectives

Many attempts are being made in applying AIDD. The results need to be judiously applied. The AIDD models are only as good as the training provided to them. If sufficient reliable data is provided during training, we can trust the model. Hence, it is important to pay attention to data quality before taking up the AI model development. It has been a roller-coaster ride for the AIDD in the recent past, hopefully the trend will stabilize soon and the AIDD techniques will be adopted by all drug discovery scientists.

## References


1. McCarthy, J. Artificial intelligence, logic and formalizing common sense. In Philosophical logic and artificial intelligence;Springer: 1989, pp 161-190.
2. McCarthy, J., From here to human-level AI. Artificial Intelligence2007, pp 1174-1182.
3. https://www.ibm.com/in-en/cloud/learn/what-is-artificial-intelligence
4. Bharatam, P. V. Computer-aided drug design. In Drug Discovery and Development;Springer: 2021, pp 137-210.
5. Zhang, Y.; Rajapakse, J. C., Machine learning in bioinformatics. John Wiley & Sons, Inc.; New Jersey, US: 2009; Vol. 4.
6. Zupan, J.; Gasteiger, J., Neural networks in chemistry and drug design. John Wiley & Sons, Inc.; New Jersey, US: 1999.
7. Sterling, L.; Shapiro, E. Y., The art of Prolog: advanced programming techniques.MIT press, Cambridge, MA: 1994.
8. Tanimoto, S. L., The elements of artificial intelligence: an introduction using LISP.Computer Science Press, Inc.; New York, US: 1987.
9. Gertrudes, J. C.; Maltarollo, V. G.; Silva, R.; Oliveira, P. R.; Honorio, K. M.; Da Silva, A., Machine learning techniques and drug design. Current Medicinal Chemistry 2012, 19, 4289-4297.
10. Lo, Y.-C.; Rensi, S. E.; Torng, W.; Altman, R. B., Machine learning in chemoinformatics and drug discovery. Drug Discovery Today 2018, 23, 1538-1546.
11. Mak, K.-K.; Pichika, M. R., Artificial intelligence in drug

development: present status and future prospects. Drug Discovery Today 2019, 24, 773-780.

12. Narayanan, A.; Keedwell, E. C.; Olsson, B., Artificial intelligence techniques for bioinformatics. Applied Bioinformatics 2002, 1, 191-222.
13. Lavecchia, A., Machine-learning approaches in drug discovery: methods and applications. Drug Discovery Today 2015, 20, 318-331.
14. Suzuki, E.; Akutsu, T.; Ohsuga, S., Knowledge-based system for computer-aided drug design. Knowledge-Based Systems 1993, 6, 114-126.
15. Zhu, Y.; Elemento, O.; Pathak, J.; Wang, F., Drug knowledge bases and their applications in biomedical informatics research. Brief. Bioinfo. 2019, 20, 1308-1321.
16. Whirl-Carrillo, M.; McDonagh, E. M.; Hebert, J.; Gong, L.; Sangkuhl, K.; Thorn, C.; Altman, R. B.; Klein, T. E., Pharmacogenomics knowledge for personalized medicine. Clin. Pharmacol. Ther. 2012, 92, 414-417.
17. https://dailymed.nlm.nih.gov/dailymed/
18. Hecker, N.; Ahmed, J.; von Eichborn, J.; Dunkel, M.; Macha, K.; Eckert, A.; Gilson, M. K.; Bourne, P. E.; Preissner, R., SuperTarget goes quantitative: update on drug-target interactions. Nucleic Acids Research 2012, 40, D1113-D1117.
19. Ayvaz, S.; Horn, J.; Hassanzadeh, O.; Zhu, Q.; Stan, J.; Tatonetti, N. P.; Vilar, S.; Brochhausen, M.; Samwald, M.; Rastegar-Mojarad, M., Toward a complete dataset of drug-drug interaction information from publicly available sources. J. Biomed. Infor.2015, 55, 206-217.
20. Zeng, X.; Tu, X.; Liu, Y.; Fu, X.; Su, Y., Toward better drug discovery with knowledge graph. Curr. Opin. Struct. Biol.2022, 72, 114-126.
21. Ebejer, J.-P.; Finn, P. W.; Wong, W. K.; Deane, C. M.; Morris, G. M., Ligity: A non-superpositional, knowledge-based approach to virtual screening. J. Chem. Inf. Model. 2019, 59, 2600-2616.
22. Huang, S.-Y.; Grinter, S. Z.; Zou, X., Scoring functions and their evaluation methods for protein-ligand docking: recent advances and future directions. Phys. Chem. Chem. Phy. 2010, 12, 12899-12908.
23. Velec, H. F.; Gohlke, H.; Klebe, G., DrugScore$^{CSD}$ knowledge-based scoring function derived from small molecule crystal data with superior recognition rate of near-native ligand poses and better affinity prediction. J. Med. Chem. 2005, 48, 6296-6303.
24. Huang, S. Y.; Zou, X., An iterative knowledge-based scoring function for protein-protein recognition. Proteins: Struct. Funct. Bioinfo.2008, 72, 557-579.
25. Ghose, A. K.; Herbertz, T.; Salvino, J. M.; Mallamo, J. P., Knowledge-based chemoinformatic approaches to drug discovery. Drug Discovery Today 2006, 11, 1107-1114.
26. Devillers, J., Genetic algorithms in molecular modeling. Academic Press, Inc.; San Diego, CA: 1996.
27. Douguet, D.; Thoreau, E.; Grassy, G., A genetic algorithm for the automated generation of small organic molecules: drug design using an evolutionary algorithm. J. Comput. Aided Mol. Des.2000, 14, 449-466.
28. Perez-Castillo, Y.; Lazar, C.; Taminau, J.; Froeyen, M.; Cabrera-Pérez, M. Á.; Nowe, A., GA (M) E-QSAR: a novel, fully automatic genetic-algorithm-(meta)-ensembles approach for binary classification in ligand-based drug design. J. Chem. Inf. Model. 2012, 52, 2366-2386.
29. Sun, G.; Fan, T.; Sun, X.; Hao, Y.; Cui, X.; Zhao, L.; Ren, T.; Zhou, Y.; Zhong, R.; Peng, Y., In silico prediction of O6-methylguanine-DNA methyltransferase inhibitory potency of base analogs with QSAR and machine learning methods. Molecules 2018, 23, 2892.
30. Spiegel, J. O.; Durrant, J. D., AutoGrow4: an open-source genetic algorithm for de novo drug design and lead optimization. J. Cheminfo. 2020, 12, 1-16.
31. Goodsell, D. S.; Morris, G. M.; Olson, A. J., Automated docking of flexible ligands: applications of AutoDock. J. Mol. Reco. 1996, 9, 1-5.
32. Chu, Y.; He, X., MoleGear: a java-based platform for evolutionary de novo molecular design. Molecules 2019, 24, 1444.
33. Dey, F.; Caflisch, A., Fragment-based de novo ligand design by multiobjective evolutionary optimization. J. Chem. Inf. Model. 2008, 48, 679-690.
34. Burbidge, R.; Trotter, M.; Buxton, B.; Holden, S., Drug design by machine learning: support vector machines for pharmaceutical data analysis. Comp. Chem.2001, 26, 5-14.
35. Vamathevan, J.; Clark, D.; Czodrowski, P.; Dunham, I.; Ferran, E.; Lee, G.; Li, B.; Madabhushi, A.; Shah, P.; Spitzer, M., Applications of machine learning in drug discovery and development. Nature Reviews Drug Discovery 2019, 18, 463-477.
36. Vishnoi, S.; Matre, H.; Garg, P.; Pandey, S. K., Artificial intelligence and machine learning for protein toxicity prediction using proteomics data. Chem. Biol. Drug Design 2020, 96, 902-920.
37. Ekins, S., The next era: deep learning in pharmaceutical research. Pharm. Res.2016, 33, 2594-2603.
38. Chen, H.; Engkvist, O.; Wang, Y.; Olivecrona, M.; Blaschke, T., The rise of deep learning in drug discovery. Drug Discovery Today 2018, 23, 1241-1250.
39. Bajorath J. Data analytics and deep learning in medicinal chemistry. Future Med. Chem. 2018, 13, 1541-1543.
40. Zhang, L.; Tan, J.; Han, D.; Zhu, H., From machine learning to deep learning: progress in machine intelligence for rational drug discovery. Drug Discovery Today 2017, 22, 1680-1685.
41. Mayr, A.; Klambauer, G.; Unterthiner, T.; Hochreiter, S., DeepTox: toxicity prediction using deep learning. Front. Environ. Sci.2016, 3, 80.
42. Rodriguez-Perez, R.; Bajorath, J. r., Multitask machine learning for classifying highly and weakly potent kinase inhibitors. ACS Omega 2019, 4, 4367-4375.
43. Maltarollo, V. G.; Kronenberger, T.; Espinoza, G. Z.; Oliveira, P. R.; Honorio, K. M., Advances with support vector machines for novel drug discovery. Expert Opin. Drug Discov.2019, 14, 23-33.
44. Jiménez-Luna, J.; Grisoni, F.; Schneider, G., Drug discovery with explainable artificial intelligence. Nature Machine Intell.2020, 2, 573-584.
45. Gawehn, E.; Hiss, J. A.; Schneider, G., Deep learning in drug discovery. Mol.Infor.2016, 35, 3-14.
46. Goh, G. B.; Siegel, C.; Vishnu, A.; Hodas, N. O.; Baker, N., Chemception: a deep neural network with minimal chemistry knowledge matches the performance of expert-developed QSAR/QSPR models. arXiv preprint arXiv:1706.06689 2017.
47. Murdoch, W. J.; Singh, C.; Kumbier, K.; Abbasi-Asl, R.; Yu, B., Definitions, methods, and applications in interpretable machine learning. Proc. Natl. Acad. Sci. 2019, 116, 22071-22080.
48. Nembri, S.; Grisoni, F.; Consonni, V.; Todeschini, R., In silico prediction of cytochrome P450-drug interaction: QSARs for CYP3A4 and CYP2C9. Int. J. Mol. Sci. 2016, 17, 914.
49. Jiménez, J.; Skalic, M.; Martinez-Rosell, G.; De Fabritiis, G., K deep: protein-ligand absolute binding affinity prediction via 3d-convolutional neural networks. J. Chem. Inf. Model. 2018, 58, 287-296.
50. Jiménez-Luna, J.; Pérez-Benito, L.; Martínez-Rosell, G.; Sciabola, S.; Torella, R.; Tresadern, G.; De Fabritiis, G., DeltaDelta neural networks for lead optimization of small molecule potency. Chem. Sci.2019, 10, 10911-10918.
51. Ying, Z.; Bourgeois, D.; You, J.; Zitnik, M.; Leskovec, J., Gnnexplainer: Generating explanations for graph neural networks. Adv. Neural Inf. Process. Syst.2019, 32.
52. Lipinski, C. F.; Maltarollo, V. G.; Oliveira, P. R.; Da Silva, A. B.; Honorio, K. M., Advances and perspectives in applying deep learning for drug design and discovery.Front. Robot. AI 2019, 108.
53. Fooladi, H., Deep Learning In Drug Discovery. Towards Data Science 2020.
54. Chowdhury, M.; Sadek, A. W., Advantages and limitations of artificial intelligence. In Artificial intelligence applications to critical transportation issues. Transportation Research Board, Washington, DC: 2012, 6, 360-375.

Review Article

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# Biomarkers in COVID-19: Prospects and Problems

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COVID-19 caused by SARS-CoV-2 was declared as pandemic by WHO. After a havoc caused for a year, there was mutation in the virus resulting in different strains. SARS-CoV-2 attacks ACE2 receptors which are present not only on the lungs, but also in the various other organs causing multiorgan damage. To manifest the disease and to categorize its severity biomarkers are used as diagnostic tool. Biomarkers are biological indicators, indicating normal, diseased and variations in body function. There are three major types of biomarkers; biomarkers of susceptibility, exposure and effect. In COVID-19 viral infection numerous important biomarkers played a role in describing patients' prognosis, predictivity and accurate diagnosis. The inflammatory biomarkers include c-reactive protein, procalcitonin and creatine kinase.Blood biomarkers comprise of lymphocytes, platelet count, D-dimer. Renal damage is measured by the variation in creatinine; liver dysfunction is determined by the elevation of AST, ALT and lactate dehydrogenase. Troponin is considered as cardiac damage biomarker. The c-reactive protein is considered as a major prognostic biomarker. D-dimer predicted the chances of mortality in patients. However, development and validation of relevantbiomarkers and the correlation with clinical features remains a challenging task.

***Keywords:*** COVID-19, SARS-CoV-2, ACE2 receptors, biomarkers.

## Introduction

Corona Virus Disease 2019 (COVID-19), was declared aspandemic by World Health Organization on 11$^{th}$ March 2020. The first case was reportedin China on 31$^{st}$ December 2019 and later it spread to different parts of the world **(1)**. It is caused by Severe Acute Respiratory Syndrome (SARS) group of viruses and the causative pathogen is SARS-CoV-2. The symptoms of COVID-19 ranges from being asymptomatic to milder to severe conditions. Milder symptoms, includes fever, dry cough, dyspnoea, myalgia, sore throat and headache, and more severe and emergent manifestation includes confusion, chest pain, hypoxemia, pneumonia and other complications requiring intensive care unit (ICU) admission. These differences can also be caused by variations between countries, in the number of people tested, characteristics of the local healthcare system, the timely actions taken to containthe virus, characterizing the subtypes of thevirus, as well as the socioeconomic conditions, ethnic, geographical, and social determinants of health infrastructure. The following risk factors are associated with COVID-19, such as advanced age, obesity, male gender, heart diseases, diabetes and immunodeficiency, ethnicity/race. COVID-19 is considered as a multi-organ disease and can affect theCardiovascular System (CVS), Central Nervous System (CNS) and Gastrointestinal System (GIT). So appropriate biomarkers for the proper diagnosis, predicting the severity as well as the prognosis of the disease are necessary. Basically, biomarkers are an indicator of normal biological, pathogenic processes or response to any extraneous chemical agents in the living system **(1)**. Biomarkers can reveal the entire spectrum of disease condition from the earliest manifestations to the progression leading to the terminal stage **(2)**.

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## SARS-CoV-2

Initially there were six strains of the virus, the spread started with L strain in December, 2019and later in early 2020 there was an emergence of S strain **(3)**. By the mid 2020 there were V and G strains, the G strain was the most widespread and it mutated into GR and GH strains **(4)**. Towards the end of the year 2020 there were emergence of potentially damaging and actively spreading variants. Thevariant, B.1.1.7 carried large number of mutations and was considered to be highly infectious. The B.1.351, acquired mutation called E484K that is responsible for the alteration in the shape of a key part of the coronavirus spike protein that helps it to evade the antibodies effective against other variants. The P.1variant has 17 different mutations, including mutations in receptor binding domain of the spike protein **(5)**. The second wave of COVID-19 by the strike of B.1.617 strain, commonly called as double mutant strain **(6)**. The recent strike by the new strain, B.1.1.529 named as Omicron is a variant of concern and spread more easily than the original SARS-CoV-2 virus. This variant is hitting various parts of the world including India, triggering it to an experience of third wave of COVID-19. Respiratory cells are most likely to get attacked by coronavirus infection because of the

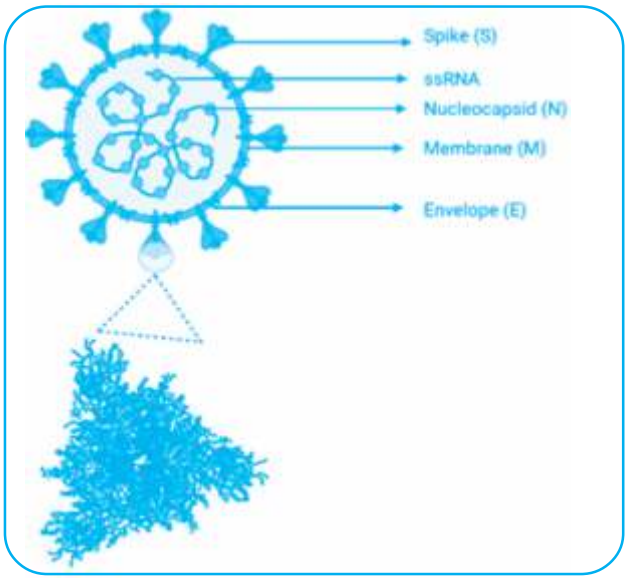


**Figure 1:** Structure of SARS-CoV-2: The SARS-CoV-2 consists of four main structural proteins; spike (S-) glycoprotein, membrane (M-) glycoprotein, envelope (E-) glycoprotein, and nucleocapsid (N) protein.

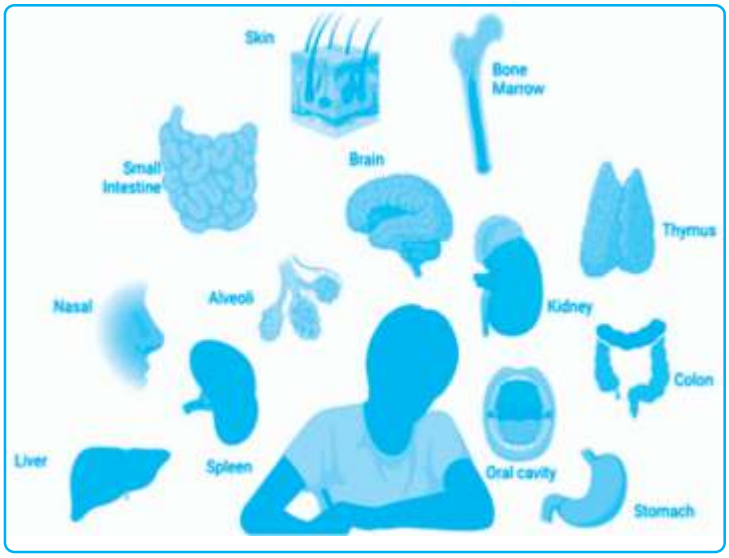


**Figure 2:** Distribution of ACE2 receptors in human body: ACE2 receptors are widely distributed in the human body particularly in the type II alveolar region. It is also present in lungs, heart, nose, kidney, intestine and brain.

expression of ACE2 receptors, which engage the viral S-protein **(Figure 1) (7,8)** and the endothelial cell surface protein TMPRSS2to facilitate the entry of the virus inside the cell **(9,10).** In lungs ACE2is mainly distributed in type II alveolar region. This indicated that the higher the ACE2 or its expression level, the higher the COVID-19 risk.The wide distribution of ACE2 receptors in the body significantly indicates the multifaceted infection of SARS-CoV-2; affecting the cardiovascular, gastrointestinal and even the central nervous systems, hence patients experience different symptoms **(Figure 2).** A new study carried out in 3D models showed that SARS-CoV-2 could infect organoid cells not only derived from the airway system, but also the gut. The virus enters the host cell via the spike protein, which adheres to the human ACE2 receptor through its receptor binding site **(11,12).** (**Figure 3)** provides an insight of the entry of virus into the host cell.

## Biomarkers and COVID-19

Biomarker is a characteristic that can be objectively measured and evaluated as an indicator of normal biological process, pathogenic condition or pharmacological response to any therapeutic stimulation **(13).** It can indicate various disease characteristics including the level and type of exposure to environmental factor, genetic susceptibility, and the genetic responses to environmental changes **(Figure 4).** Once a biomarker is validated it can be used to assess the disease risk in general population or even to confirm the diagnosis in patients. The severity of the COVID-19 has created the urge of biomarkers; which can detect the development and progression of disease in patients.According to the GlobalBiomarkers Database, a large number of different biomarkers have been utilized for COVID-19 trials for different purposes,but few of them are validated for clinical application, with the risk that the results produced are not reliable and are not of much use for clinicaldecision making.

As per the pathogenesis of the disease after the entry of the virus in the lung cells via ACE2 receptors, there is release of proinflammatory cytokines and inflammation. Initially this inflammation may be seen to have protective role but develops cellular and tissue lesions later **(14).** Hyperinflammation in systemic region leads to vascular lesions and thereby affects the thrombocytes, cytokines and develop multisystemic lesions. This phenomenon provides the opportunity to develop different biomarkers.As per the variation in disease severity and the possibilities of asymptomatic individuals it remains a challenge to develop validated biomarkers with clinical importance. After 5-14 days of infection there is an immunological response and the person starts to experience symptoms. The neutralizing antibodies arereleased after the responseto the target receptor binding domain of the spike protein of virus. There is an upsurge in the proteins such as azurocidin, cathepsin, ceruloplasmin, gamma-enolase, gap junction delta-2 protein, hemopexin, immunoglobulin heavy constant alpha 1, histone protein, immunoglobulin kappa light chain, immunoglobulin heavy constant mu, myeloblastin, myeloperoxidase, neutrophil elastase, transketolase, transcobalamin-, and vitronectin with decrease in tubulin alpha-1C chain in SARS-CoV-2 infected individuals **(15).** The exacerbated protein response can serve as the biomarkers in COVID-19 infection.

## Different Biomarkers of COVID-19

The disease severity of COVID-19 is associated with the presence of inflammation and huge surge of cytokines. This is characterized by anincreased interferon-$\alpha$, interleukin-2, tumor necrosis factor-$\alpha$, interferon-inducible protein 10 and macrophage inflammatory protein 1-$\alpha$. Patients with fatality has shown marked increase in

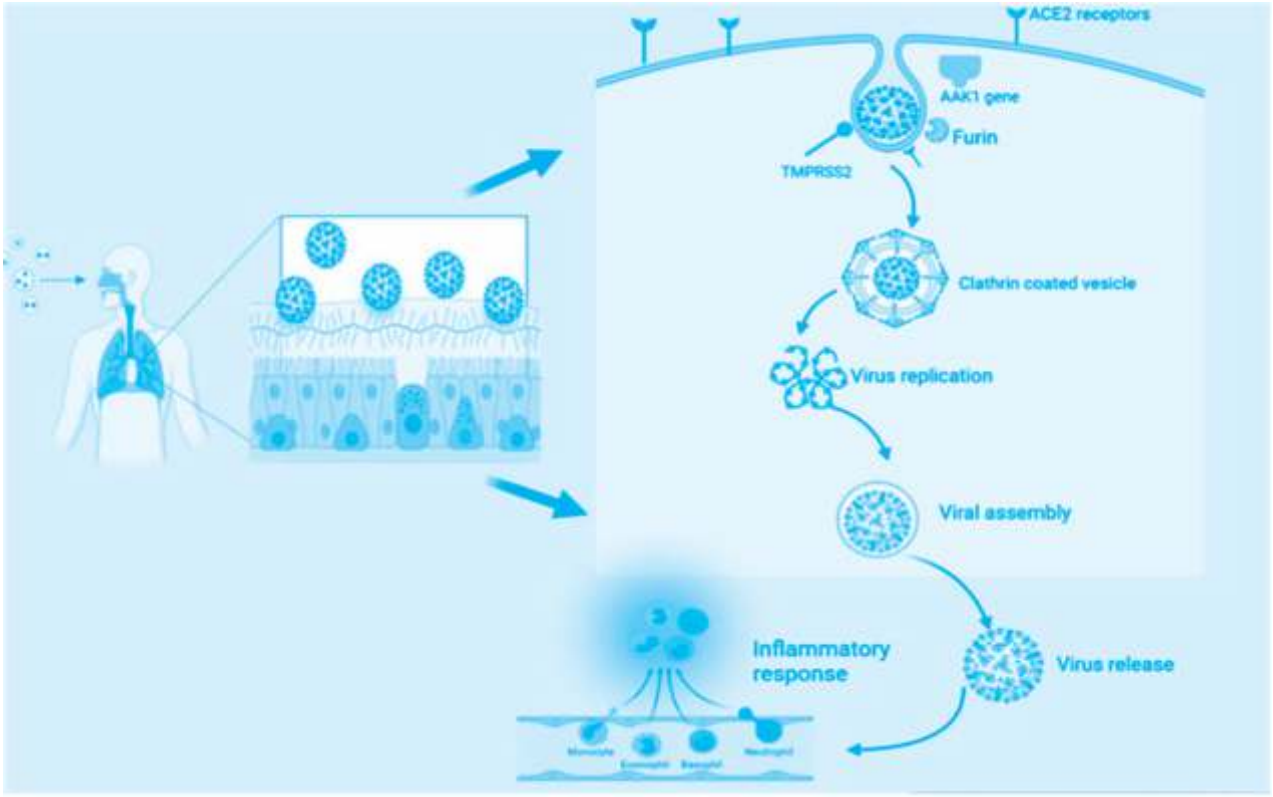


**Figure 3:** Mechanism of adhesion and entry of SARS-CoV-2: The virus paves its path to the lungs via spike protein which binds to ACE2 receptors with the help of two interacting proteins;furin and TMPRSS2. TMPRSS2 cleaves the viral S glycoprotein and initiate viral activation. Then the virus gets enclosed in clathrin-coated vesicle and replicates inside the cell. Afterreplication the virus releases from the cell to attack other cells and the inflammatory response initiates.

ferritin. To predict the disease severity biomarkers used are neutrophils, lymphocytes, C-reactive proteinan indicator ofinflammatory condition, and D-dimer an indicatorofblood clotting status **(14).** In adults, neutrophils, lymphocytes, C-reactive protein, and D-dimer are used to predict the disease severity. Lymphocyte count, C-reactive protein, lactate dehydrogenase can be used as biomarkers for predicting the susceptibility of mortality. In severely affected patients there was a marked increase in IL-6, IL-2, IL-4, IL-10, TNF-á; whereas IFN-ã remained within the normal limits **(16,17,18).** Lymphopenia is also considered as the marker of disease severity **(19,21).** As SARS-CoV-2 affects cardiovascular system, which induced hypotension and tachycardia,elevated levels of cardiac biomarkers such as troponin I and BNP can provide evidence forthe involvement of CVS **(15).** The association between inflammation and thrombosis involves the endothelial cell activation and the platelet activation as well as the proinflammatory mediators. Even though routine biochemical assays do not provide complete descriptive knowledge about the disease severity, they appear to give the likelihood of the current status and theprogression of the disease.Protein biomarkers enhance the evidence of the extent of infection and other

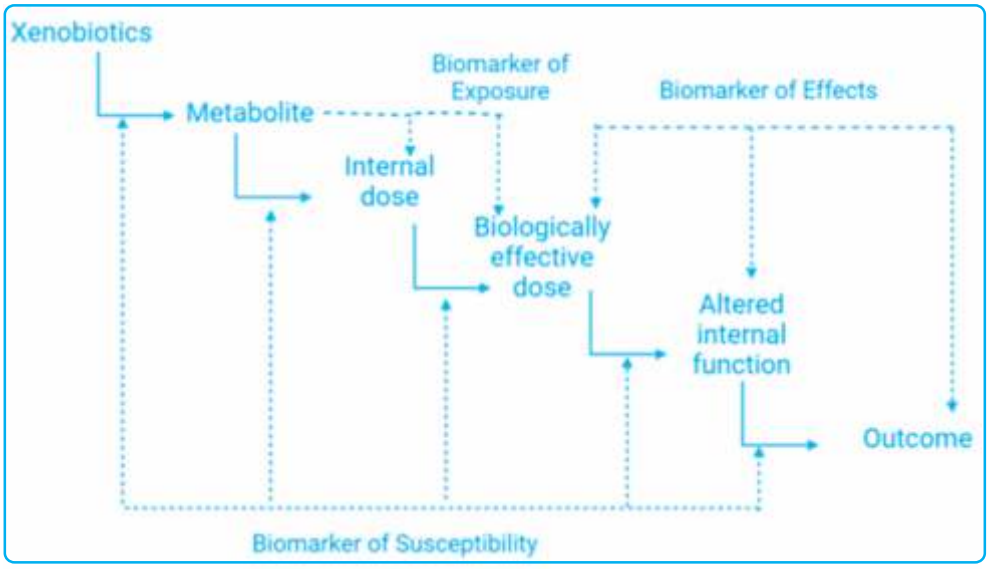


**Figure 4:** Flowchart of different classes of biomarkers: The three major types of biomarkers are biomarkers of susceptibility (environmental factors interact with individuals), biomarkers of exposure (an external chemical, xenobiotics, or metabolite interact with the cells), and biomarkers of effects (represent the measurable changes of adversity due to exposure of external chemicals).

immunological parameters such as IgG, IgM that canprovide a wider view about the possibility of chronic effects and the treatment choices during the acute phase of infection **(2,21).**

## Lymphocyte count

It has been observed that there is a decrease in lymphocytes count in Covid 19 patients **(21).** This decrease in lymphocyte count can facilitate the increase in neutrophil tolymphocyte ratio, being considered as one of the prominent biomarkers for distinguishing between the severe and non-severe patients. However, it has been observed that during the course of treatment, the ratio could be disrupted and the exact state of the disease would not be established.

## Platelet count

It is a simple, cheap and rapidly adopted biomarker for COVID-19 patients.Studies have reported that low platelet count has been associated with an increased risk of severity and mortality for COVID-19 patients. The patients with significant lower platelet count and elevated immature platelet fraction (IPF) during treatment had longer average hospitalization stay. Lung tissue damage and pulmonary endothelial cells may activate platelets, resulting in the aggregation and formation of microthrombi and thereby increase the platelet consumption. Study indicated thatthrombocytopenia was more prominent in non survivors than survivors during the time of admission of patients **(21,23).**

## C-Reactive Protein (CRP)

The elevated levels of CRP (>10 mg/l) are seen in the infected patients. Studies have shown that CRP is the first biomarker that changes during the perturbations in the physiologicalcondition. Hence, CRP can be used to predict the COVID 19 disease severity **(21).** CRP, can alsobe used to detect the hyper inflammation condition. However, the Erythrocyte Sedimentation Rate (ESR) islower than CRP during the early phase of severe cases and hence can be used as a sensitive biomarker for the disease prediction **(24).**

## Procalcitonin (PCT)

Procalcitoninis another important and crucial biomarker for predicting the disease severity. It is a glycoprotein produced in the C- cells of the thyroid gland. Under normal condition, the level (0.1 ng/ml) is undetectable and increased(>0.5 ng/ml) during COVID-19 infection. During severe infection (bacterial, parasitic, and fungal) with systemic manifestations, PCT level may rise to over 100 ng/ml, produced mostly by extra-thyroid tissue.Although PCT value may be helpful initially in the determination of the severity of illness, but it is not a reliable prognostic indicator **(25).**It may be influenced by preexisting comorbid conditions, such as chronic kidney disease and congestive heart failure.The baseline values observed in these cases generally very high **(26).**

## Creatine kinase (CK)

Patients infected with SARS-CoV-2 is seen to have increased creatine kinase. Elevation in CK is an indication of muscle damage and hence serve as a useful biomarker. The mechanism of this elevation and damage to muscle is not well understood, however during viral infection there is viral mediated invasion and damage to the myocytes. Further, this process can also be arisingdue to thehyper inflammation caused by anincreased cytokine storm. The viral antibodies get deposited in the muscles which can damage to the myocyte **(23).**

There are various other metabolic biomarkers which exists during the infection with SARS-CoV-2 virus.

## *Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT)*

AST and ALT are the markers of hepatotoxicity, and the underlying cause of liver damage due to COVID-19 is not well understood. The SARS-CoV-2 virus attacks the ACE2 receptors present on the liver and bind to cholangiocytes and perturb the liver function. The inflammatory response due to lymphocytes and macrophages initiates cytokine storm during viral infection, and also affects the pulmonary function. The clinical relevance of abnormal liver function test is limited and hence utilization of AST, ALT as biomarkers for SARS-CoV-2 infection is unclear **(27).**

## Creatinine

The abnormality in the kidney function results in an increase of creatinine and Blood Urea Nitrogen (BUN). An increase in biomarkers of kidney function is seen in patients suffering from COVID-19. The hypothesis postulated is that there is a spread and build-up of virus in the kidney resulting in renal necrosis **(23).** Creatininelevel wassignificantly increased during the mortality and therefore it can be a relevant prognostic biomarker.

## D- dimer

D-dimer is the product of cross-linked fibrin that is present in blood during blood clotting **(28).** Patients suffering from severe corona virus infection is seen with increased D-dimer level (36-43%) and other coagulation factors **(14).** The elevated D-dimer level indicates an increased coagulation and fibrinolysis condition **(21).** This biomarker more prominently increased in patients with COVID-19 than in the patients suffering only from pneumonia. Along with CRP, D-dimer is an important biomarker of inflammation. The non-survivors have demonstrated with an increase in D-dimer level and hence considered as a useful prognostic biomarker. Anticoagulant regimens display a decreases in D-dimer level, suggesting a better outcome in COVID-19 patients **(23,29).**

## Lactate dehydrogenase (LDH)

LDH level increased during cell necrosis, and served as an indicator oflungs damage due to SARS-CoV-2. The LDH levels are high in ICU patients than in non-ICU,which can infer that lactate dehydrogenase can beused as a predictive biomarkerin COVID-19 infection **(21).**

## Troponin-I

The COVID-19 infected patients have seen with an increased risk of cardiovascular disorders. High-sensitivity

cardiac troponin I (hs-TnI) is a biomarker of disease progression and mortality. Troponin I levels are significantly high in severe COVID-19 patients **(14,21).** This can be used as a biomarker of cardiac damage in COVID-19 infection.

## Emerging Clinical Biomarkers in COVID-19

There are recent advancements in the biomarkers of COVID-19 and it is emerging day-by-day. The miRNAs are the key players in several biological processes that regulate the differentiation, development and activation of immune cells in both innate and adaptive immunity. The miRNAs have the potential to be used as diagnostic and therapeutic biomarkers. However, discovery and validation are essential for improving the diagnosis of infection and the clinical monitoring in COVID-19, before these are established as a valid biomarker **(30).** Decreased serum level of sphingosine-1-phosphate: a novel predictor of clinical severity in COVID-19 can help to evaluate the severity/mortality of COVID 19 patients **(31).** Several lines of evidences signal a probable link between the gut microbiota and the host's immune response in COVID-19. Although still in a nascent stage, efforts are being made to establish a link between the complex immunological crosstalk and the microbiome's potential as a biomarker and therapeutic target in COVID-19 infection **(32).**

## Challenges in Biomarker Development

Developing different categories of biomarkers is a challenging task and an incredible effort have been made in developing them. From the beginning of the development such as selection and isolation of body sample, use of various techniques to validate the same and finally the approval by the regulatory authorities hasits own difficulty and challenges **(33).** To ascertain the usefulness of the biomarkers listed in this review as indicators of disease progression, and whether they definitively rise in COVID-19 requires further data information as well as validation **(34).** The collection of the sample and its processing as well as the storage has to adopt a uniform procedure. Disease heterogenicity, the severity of individuals towards a particular disease, the genetic factors, previous history of disease prevalence, co-infections, hormone related variability are the important aspects to be considered, when biomarkers are used in clinicalrisk assessment process **(34,36).** Focusing on the technical limitations; lengthy experimental procedures, lack of uniformity in the data acquisition, reproducibility of results should be taken into consideration when biomarkers are developed. Currently, biomarker discovery is based on using the omics and high throughput technologies **(37,38).** Universally approved guidelines are not available due to compromise in quality control standards **(39,40,41).**

In summary, as the vaccine efficacy for COVID-19 treatment is very limited, rapid and an early diagnosis is imperative in providing timely health measures as well as to reduce the risk of health complications. Biomarkers played an important role in providing progression, diagnosis, and the predictability of disease occurrence. Biomarkers also provides with plethora of information regarding the severity of disease condition, which enable the healthcare workers to provide a right medical treatment at an earliest possible time.

Although the biomarkers play a crucial role in the detection, prognosis and severity of the disease, the patients after recovery may experience reinfection of the disease and this would require more specific biomarker, as general inflammatory biomarkers will not play a role in this case. The rates of evolution and mutations in coronaviruses are very high, which makes it difficult to identify and develop a specific biomarker against them.Hence, there is a need of more specific and validated biomarkers which will predict the recurrence, severity, and specificity of the disease. Finally, the application and combination of all these biomarkers in detection, diagnostics, treatment and prevention will be the ultimate weapon to win the war against COVID-19.

## References


1. Caruso FP, Scala G, Cerulo L, Ceccarelli M. A review of COVID-19 biomarkers and drug targets: resources and tools. Brief Bioinform. 2020:1-13.
2. Whetton AD, Preston GW, Abubeker S, Geifman N. Proteomics and Informatics for Understanding Phases and Identifying Biomarkers in COVID-19 Disease. Vol. 19, Journal of Proteome Research. 2020:19(11):4219-4232.
3. Wang MY, Zhao R, Gao LJ, Gao XF, Wang DP, Cao JM. SARS-CoV-2: Structure, Biology, and Structure-Based Therapeutics Development. Front Cell Infect Microbiol. 2020;10(November):1–17.
4. Mercatelli D, Giorgi FM. Geographic and Genomic Distribution of SARS-CoV-2 Mutations. Front Microbiol. 2020;11:2020–2.
5. Martin Webb L, Matzinger S, Grano C, Kawasaki B, Stringer G, Bankers L, et al. Identification of and Surveillance for the SARS-CoV-2 Variants B.1.427 and B.1.429 — Colorado, January–March 2021. MMWR Morb Mortal Wkly Rep. 2021;70(19):717–8.
6. Thompson CN, Hughes S, Ngai S, Baumgartner J, Wang JC, McGibbon E, Devinney K, Luoma E, Bertolino D, Hwang C, Kepler K, Del Castillo C, Hopkins M, Lee H, DeVito AK, Rakeman JL; PhD1, Fine AD. Rapid Emergence and Epidemiologic Characteristics of the SARS-CoV-2 B.1.526 Variant - New York City, New York, January 1-April 5, 2021. MMWR Morb Mortal Wkly Rep. 2021 May 14;70(19):712-716. doi: 10.15585/mmwr.mm7019e1.
7. Cleary SJ, Pitchford SC, Amison RT, Carrington R, Robaina Cabrera CL, Magnen M, et al. Animal models of mechanisms of SARS-CoV-2 infection and COVID-19 pathology. Br J Pharmacol. 2020;177(21):4851–65.
8. Kai H, Kai M. Interactions of coronaviruses with ACE2, angiotensin II, and RAS inhibitors—lessons from available evidence and insights into COVID-19. Hypertens Res [Internet]. 2020;43(7):648–54. Available from: http://dx.doi.org/10.1038/s41440-020-0455-8
9. Zhang L, Guo H. Biomarkers of COVID-19 and technologies to combat SARS-CoV-2. Adv Biomark Sci Technol [Internet]. 2020;2:1–23. Available from: https://doi.org/10.1016/j.abst.2020.08.001
10. Mollica V, Rizzo A, Massari F. The pivotal role of TMPRSS2 in coronavirus disease 2019 and prostate cancer. Futur Oncol. 2020;16(27):2029–33.
11. Rossi GA, Sacco O, Mancino E, Cristiani L, Midulla F. Differences and similarities between SARS-CoV and

SARS-CoV-2: spike receptor-binding domain recognition and host cell infection with support of cellular serine proteases. Infection [Internet]. 2020;48(5):665–9. Available from: https://doi.org/10.1007/s15010-020-01486-5

12. Barash A, Machluf Y, Ariel I and Dekel Y (2020) The Pursuit of COVID-19 Biomarkers: Putting the Spotlight on ACE2 and TMPRSS2 Regulatory Sequences. Front. Med. 7:582793. doi: 10.3389/fmed.2020.58279313. David Crawford E, Ventii K, Shore ND. New biomarkers in prostate cancer. Oncol (United States). 2014;28(2):303–22.
14. Danwang C, Endomba FT, Nkeck JR, Wouna DLA, Robert A, Noubiap JJ. A meta-analysis of potential biomarkers associated with severity of coronavirus disease 2019 (COVID-19). Vol. 8, Biomarker Research. 2020.8:37,doi: 10.1186/s40364-020-00217-0.
15. Ward JB, Henderson RE. Identification of needs in biomarker research. Environ Health Perspect. 1996;104(SUPPL. 5):895–900.
16. Kreutmair S, Unger S, Núñez NG, Ingelfinger F, Alberti C, De Feo D, et al. Distinct immunological signatures discriminate severe COVID-19 from non-SARS-CoV-2-driven critical pneumonia. Immunity [Internet]. 2021; Available from: https://doi.org/10.1016/j.immuni.2021.05.002
17. Dugan HL, Stamper CT, Li L, Changrob S, Asby NW, Halfmann PJ, et al. Profiling B cell immunodominance after SARS-CoV-2 infection reveals antibody evolution to non-neutralizing viral targets. Immunity [Internet]. 2021; Available from: https://doi.org/10.1016/j.immuni.2021.05.001
18. Lu Q, Liu J, Zhao S, Gomez Castro MF, Laurent-Rolle M, Dong J, et al. SARS-CoV-2 exacerbates proinflammatory responses in myeloid cells through C-type lectin receptors and Tweety family member 2. Immunity [Internet]. 2021; Available from: https://doi.org/10.1016/j.immuni.2021.05.006
19. Xiang J, Wen J, Yuan X, Xiong S, Zhou X, Liu C, et al. Potential biochemical markers to identify severe cases among COVID-19 patients. BMJ; 2020;19:1–10.
20. Hosseini A, Hashemi V, Shomali N, Asghari F, Gharibi T, Akbari M, et al. Innate and adaptive immune responses against coronavirus. Biomed Pharmacother. 2020;132:1–16.
21. Kermali M, Khalsa RK, Pillai K, Ismail Z, Harky A. The role of biomarkers in diagnosis of COVID-19 - A systematic review. Life Sci. 2020 Aug 1;254:117788. doi: 10.1016/j.lfs.2020.117788. Epub 2020 May 13.
23. Malik P, Patel U, Mehta D, Patel N, Kelkar R, Akrmah M, et al. Biomarkers and outcomes of COVID-19 hospitalisations: systematic review and meta-analysis. BMJ Evidence-Based Med. 2021 Jun;26 (3):107-108.
24. C reactive protein correlates with CT findings and predicts severe COVID 19 early - Tan - - Journal of Medical Virology - Wiley Online Library [Internet]. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/jmv.25871
25. Schuetz P. Procalsitonin for Diagnosis of Infection and Guide to Antibiotic Decision. BMC Med J. 2011;107:1–9.
26. Giovanni Ponti, Monia Maccaferri, Cristel Ruini, Aldo Tomasi & Tomris Ozben (2020) Biomarkers associated with COVID-19 disease progression, Critical ReviewsinClinical LaboratorySciences, 57:6, 389-399, DOI: 10.1080/10408363.2020.1770685
27. Musa S. Hepatic and gastrointestinal involvement in coronavirus disease 2019 (COVID-19): What do we know till now? Arab J Gastroenterol. 2020;21(1):3–8.
28. Yu HH, Qin C, Chen M, Wang W, Tian DS. D-dimer level is associated with the severity of COVID-19. Thrombosis Research. 2020 Nov 1;195:219–25.
29. Kollias A, Kyriakoulis KG, Dimakakos E, Poulakou G, Stergiou GS, Syrigos K. Thromboembolic risk and anticoagulant therapy in COVID-19 patients: emerging evidence and call for action. Br J Haematol. 2020;189(5):846–7.
30. A. Guterres, C.H. de Azeredo Lima, R.L. Miranda, et al., What is the potential function of microRNAs as biomarkers and therapeutic targets in COVID-19?, Infection, Genetics and Evolution (2019), https://doi.org/10.1016/j.meegid.2020.104417
31. Marfia G, Navone S, Guarnaccia L, Campanella R, Mondoni M, Locatelli M, Barassi A, Fontana L, Palumbo F, Garzia E, Ciniglio Appiani G, Chiumello D, Miozzo M, Centanni S, Riboni L. Decreased serum level of sphingosine-1-phosphate: a novel predictor of clinical severity in COVID-19. EMBO Mol Med. 2021 Jan 11;13(1):e13424. doi: 10.15252/emmm.202013424. Epub 2020 Dec 9. PMID: 33190411; PMCID: PMC7744841.
32. Hussain, I., Cher, G., Abid, M. A., & Abid, M. B. (2021). Role of Gut Microbiome in COVID-19: An Insight Into Pathogenesis and Therapeutic Potential. *Frontiers in immunology*, *12*, 765965. https://doi.org/10.3389/fimmu.2021.765965
33. Davis KD, Aghaeepour N, Ahn AH, Angst MS, Borsook D, Brenton A, et al. Discovery and validation of biomarkers to aid the development of safe and effective pain therapeutics: challenges and opportunities. Nat Rev Neurol [Internet]. 2020;16(7):381–400. Available from: http://dx.doi.org/10.1038/s41582-020-0362-2
34. Samprathi M and Jayashree M (2021) Biomarkers in COVID-19: An Up-To-Date Review. *Front. Pediatr.* 8:607647. doi: 10.3389/fped.2020.607647
35. Gupta S, Venkatesh A, Ray S, Srivastava S. Challenges and prospects for biomarker research: A current perspective from the developing world. Biochim Biophys Acta - Proteins Proteomics. 2014;1844(5):899–908.
36. Weaver T, Maurer J, Hayashizaki Y. Sharing genomes: An integrated approach to funding, managing and distributing genomic clone resources. Nat Rev Genet. 2004;5(11):861–6.
37. Amur S, Lavange L, Zineh I, Buckman-Garner S, Woodcock J. Biomarker qualification: Toward a multiple stakeholder framework for biomarker development, regulatory acceptance, and utilization. Clin Pharmacol Ther. 2015;98(1):34–46.
38. Food and Drug Administration C for DE and R (CDER). Guidance for Industry and FDA Staff: Qualification Process for Drug Development Tools. Food Drug Adm [Internet]. 2014;(October):1–32. Available from: http://www.fda.gov/cder/guidance/index.htm%0Ahttp://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm230597.pdf
39. Weiss J, Hoffmann U, Aerts HJWL. Artificial intelligence-derived imaging biomarkers to improve population health. Lancet Digit Heal [Internet]. 2020;2(4):e154–5. Available from: http://dx.doi.org/10.1016/S2589-7500(20)30061-3
40. Mayeux R. Biomarkers: Potential Uses and Limitations. NeuroRx. 2004;1(2):182–8.
41. De Bock M, De Seny D, Meuwis MA, Chapelle JP, Louis E, Malaise M, et al. Challenges for biomarker discovery in body fluids using SELDI-TOF-MS. J Biomed Biotechnol. 2010;2010.

Review Article

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# The six challenges to pharmacy practice in India

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The Indian pharmaceutical industry has made significant contribution to the country and very correctly, India is called as "pharmacy of the world". However, in the last 90 years, this has happened at the cost of practice of pharmacy. The best quality medicines used inappropriately may lead to failure of achieving the desired outcomes.

This commentary outlines the six challenges to the growth of pharmacy practice in India. An attempt has been made to detail the challenge and offer a practical solution to overcome the challenge. It would form an interesting reading for those who see the practice from the periphery and, often, come up with theoretical solutions.

While inaugurating the first Global Innovation Summit of the pharmaceuticals sector on Thursday, November 18, 2021, the Prime minister of India said "We have exported life-saving medicines and medical equipment to over 150 countries during the initial phase of the pandemic. We have also exported more than 65 million doses of CoViD vaccines to nearly 100 countries this year". This acknowledgement by the Prime minister not only underlines the fact that "India is the pharmacy of the world" but also reflects the trust in the Indian pharmaceutical industry.

The Indian pharmaceuticals market is dominated by generic drugs which constitute nearly 70% of the market, whereas over the counter (OTC) medicines and patented drugs make up to 21% and 9%, respectively. In August 2021, export of drugs & pharmaceuticals stood at US$ 2.0 billion as compared to US$ 1.97 in August 2020.[1] These statistics might appear impressive to the naïve. However, those who are in the pharmaceutical sector know well that the India has one of the lowest manufacturing costs in the world. It is lower than that of USA and almost half of Europe. This edge has been created by the pharmaceutical industry who have relied upon the rich educational background and the vast potential of the graduates and post-graduates coming out of the pharmacy schools in the country.

The first pharmacy college in the world - 'College de Pharmacie' - was established in Paris in 1777. In America, the Philadelphia College of Pharmacy was founded in 1821 followed by Massachusetts College of Pharmacy in 1823 and New York College of Pharmacy in 1829. India is one of the latecomers in the area of pharmacy education. The pharmacy education in India was started in Banaras Hindu University in 1932 by late ML Schroff, better known as "Father of Pharmacy in India". His initiative was well received by none other than Pandit Madan Mohan Malaviya, a national figure and vice chancellor of the Banaras Hindu University. In July 1937, the course was re-named as B.Pharm.; and, in April 1940, the first M. Pharm. course (as a research degree) was started in BHU.[2]

The available records confirm that before India gained independence in 1947, there were only 3 institutions offering pharmacy degree programs. As of a conservative estimate, the total number of pharmacy institutions in the countries stands over 2000 in 2021.

The gain in the strength of the Indian pharmaceutical industry has been at the cost of pharmacy practice. Simply put, given the role models and the plethora of available job opportunities, the college pass outs had chosen to work in the industry. A very limited handful had opted for the pharmacy practice. This writing is a viewpoint detailing the six challenges to the growth of pharmacy practice in the country and offers some practical solutions as well.

It is important to realize that using a high quality drug product does not necessarily mean that the patients get better treatment(s). Using them 'correctly' is a major determinant for achieving the desired outcomes.

There are multiple real time examples of taking enteric coated tablet with or after food, thereby defying the whole purpose of the coating. And, while this could be happening around, the manufacturer is certainly not to be blamed. Largely, the sale and dispensing of medicines in the community is in the hands of freshers who 'gain' experience while at work. Hence, the role of the practicing pharmacists cannot be overlooked.

Pharmacy Practice is the discipline of pharmacy which involves developing the professional roles of pharmacists. Practicing pharmacists work in wards/ ICUs of a hospital,

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hospital pharmacy dispensing vertical, retail pharmacist ('chemists and druggists' on the boards one will see in the neighborhood market), drug information pharmacists, specialty pharmacists, counselors in hospitals and wellness centres. An essential component in the practice of pharmacy is owning up the responsibility of outcomes with drug or non-drug therapy. Essentially, it requires working in sync with the patients and the clinicians to ensure that patients receive medications appropriate to their needs.

NIPER, Mohali has started this discipline in 2002 after due consultation and caution. The preparation has been strong, with the first set of instructors sent to UK for a 3-week training in the various areas of practice of pharmacy. Subsequently, under the Higher Education Link programme of the DFID, managed by the British council, NIPER, Mohali received a 3 year long support to establish the department of pharmacy practice that offers M.Pharm. degree. Under this programme, the Medicine information Centre was established at NIPER, Mohali.

As of 2021, India has over 35 colleges offering 2-year M.Pharm. Degree in pharmacy practice/ hospital pharmacy/ clinical pharmacy. In contrast, there are over 250 colleges offering the 6-year Pharm.D. program.

American College of Clinical Pharmacy defines 'Clinical pharmacy as that area of pharmacy concerned with the science and practice of rational medication use'. The unabridged definition, however, is organized into three sections: the discipline of clinical pharmacy, the clinical pharmacist, and the roles of the clinical pharmacist in the health care system.[3]

A deeper look at the prevailing pharmacy practice in the country shall reveal that the pharmacy practice in the country is growing - in clusters. While the markers of service quality and performance indicators are in place for many other professions, the gap for pharmacy seems to be larger and the impact is killing. Mere availability of Good Pharmacy Practices guidelines does not necessarily translate into better quality of service.

The six issues identified to be challenges to the growth of pharmacy practice in the country are enumerated. In the subsequent paragraphs, each one of these is elaborated upon with proposed alternatives to adopt.

1. Availability of 'trained' teachers
2. Demonstration of skills
3. Networking among the institutions
4. Involvement with the community
5. Lack of robust and closer interaction with the clinicians
6. Sensitization of the younger students

## Availability of 'trained' teachers

Practice of pharmacy cannot progress without learning the skills. And, acquiring skills is not possible without training. We have, most of the times, not been able to distinguish between 'qualified' and 'trained' personnel in work areas. And, this has meant that the qualified personnel slowly acquire the functional skills. This pace is not acceptable for growth of pharmacy practice in our country.

The issue is compounded by the mushrooming growth of colleges offering B.Pharm courses. Exceptions apart, the teachers have never been into a practice environment. The treatment of subject like hospital/ clinical pharmacy is insipid and disoriented. The result is lack of clarity to the young minds on what is the practice of pharmacy. A contrast to consider is the law school where the learners have clarity on their future roles.

In academia, it is assumed that those who can teach certain subjects (clinical pharmacy, pharmacotherapeutics, EBM and so forth) will become exceptional pharmacy practice teachers/instructors. This belief has only led to creation of a pool of teachers who have completed a certain number of mandated hours of training in pharmacy practice. Essentially, it helps to meet the regulatory requirement and does not contribute to improve the quality. The author has flagged and underlined this gap in the year 2004.[4]

## Demonstration of skills

Lack of skills (identifying the pulse/ injecting insulin to a family member) is yet another important factor that keeps the pharmacy students away from the areas of practice. It is a matter of common knowledge that a pharmacy graduate can elaborate to a large length upon the pharmacology of Digoxin. What is weird is the fact that that the student may not be able to identify a case of cyanosis in the neighborhood/ workplace. And, we are not talking about more serious events like angina attack, CHF or stroke. Personal experiences reaffirm the belief in the capability of the diploma in pharmacy students, who do much better in such situations. The difference is solely because of the training that they undergo.

The lack of training in life-saving skills, lack of knowledge of the dosage forms, no exposure to use of some simple devices only adds to the problems.

To improve the practice of pharmacy, there is a need to build a stronger skill set at the younger level. In light of these, it is advisable that each pharmacy institution should divert their resources towards 'real-time' training of students. The starting point could be as simple as identification and/or use of hypodermic needles. This shall be easier to do with 'trained' teachers at the pharmacy schools.

## Networking among the institutions

It is to be noted that individual institutions are promoting the practice of pharmacy through established frameworks. In this context, it keeps a lot of relevance that institutions with similar interests go for networking. The informal framework networking & collaboration does not seem to be working well. And, the decision makers invest 'much more than needed' time and energies to get into the formal framework for networking.

An example in this context is the effort to conduct studies on utilization of medicines or more specifically antimicrobial agents. Several research groups/ institutions

are working on this in the country; however, the protocols adopted are divergent. The studies are excellent when viewed 'individually'. The irony is that the results do not add to wisdom on the matter. It is very much in order, at this point, to mention that World Health Organization has standardized protocols in the open domain on multiple areas.

In a country like ours, numbers are an inherent advantage. The institutions/ research groups working on a common theme need to adopt a 'common' experimental protocol so that at the completion of the study, one can -at least- understand the differences in the patterns of consumption of medicines at different locations.

The author believes that there is an intense need for research groups to work more closely.

## Involvement with the community

The choice of pharmacy graduates not to work as a practicing pharmacist is understandable, given the poor compensation and still poorer acknowledgement. In 90 years of time, the concept of 'compounder' has not faded off the minds of people in our own houses. This notion finds its roots in the fact the pharmacy students lack the basic skills, mentioned above. And, this adds to lack of identification as a pharmacist.

It has become a ritual to take out a rally across the neighborhood on the occasion of the National Pharmacy Week. On such rallies, it is pleasing to see the neat white aprons. It is only lately that some institutions have started to put up camps for the benefit of the community. The people in the community are not aliens. Their queries are very natural - what should I do if I miss my morning medicine?, What should I not eat when I am having a certain disorder?, I am diabetic, and can I replace my breakfast with something else when I am travelling or away from home?, when should I expect relief from the throat pain I am having?, Is my medicine going to cause long term side effects?, and so forth. As long as the pharmacy students are unable to answer these points with confidence and clarity, the family members shall continue to look for solutions at the retail chemist shops or use internet to find out the answers. Since the quality of information available on internet is doubtful many a times, the window of opportunity lies here.

Of special interest are the elderly patients who receive multiple medications and have all the time plus willingness to know more about the medicines they are on. As India grows, the number of elderly people is going to swell and a special cadre of 'geriatric pharmacists' is on the horizon.

## Lack of robust and closer interaction with the clinicians

In the experience of the author, nothing works better than this. The kind of expertise clinicians have is exceptional when it comes to management of disorders. There are instances when a medicine is not required at all.

The wiser pharmacists understand their domain of having expertise in pharmacokinetic principles, pharmacology of drugs and the pharmaceutical business. As long as the pharmacists maintain their boundaries, the clinicians are very comfortable working together. The pharmacists need to understand the implications of issues around ethical principles of biomedical research.[5] Which need to be adhered strictly while working with clinicians/ and human participants.

Partnering with clinicians requires perseverance. The understanding of the canvas in which they operate is equally critical. Currently, many pharmacy schools do not address this. The best route that the practicing pharmacists can take is to start as backend support to the clinical process. A word of caution here - Jumping the line and getting into interacting with patients or the care providers can cause long term damage.

## Sensitization of the younger students

With the above in view, no doubt that within India there are a variety of explanations of pharmacy practice. Someone capturing data on a set of patients and bringing out some statistics from that data-set could claim to be working in the domain of pharmacy practice. Such enthusiasm of some researchers is causing more damage to the total scenario.

It is, therefore, very much appropriate that those who have been themselves in the professional practice of pharmacy use their wisdom and judgement to sensitize the younger students at colleges and even pharmacy colleges. While PowerPoint presentations are an accepted standard for any presentation in the current times, it shall make an entirely different impact if examples from the 'real-world' setting are shared with the younger and inquisitive minds. Larger and established schools of pharmacy practice have a greater responsibility and they are well-positioned to address this.

In conclusion, it shall suffice to say that the growth and advancement of the pharmaceutical industry needs to continue. And, there is a big scope for the practice of pharmacy to improve in India.

The author understands that there could be many other factors that have a role to play. However, this viewpoint is absolutely personal and the understanding of the reader is solicited.

## REFERENCES


1. https://www.ibef.org/exports/pharmaceutical-exports-from-india.aspx#:~:text=India%20Pharma%20Exports%20and%20Advantage%20India&text=India%20has%20exported%20US%24%203.89,US%24%201.97%20in%20August%202020accessed on Nov 26, 2021
2. http://test.pharmabiz.com/PrintArticle.aspx?aid=44185&sid=9 accessed on Nov 26, 2021
3. https://www.accp.com/docs/positions/commentaries/Clinpharmdefnfinal.pdf accessed on 03 Dec 2021
4. Tiwari P. Policy planning for the emerging need of pharmacy practice educators in India. 56th Ind Pharm Cong, Kolkata, Dec 2004 (Poster presented).
5. ICMR "National Ethical Guidelines for Biomedical and Health Research Involving Human Participants" 2017, edited by RoliMathur, published by Director General. ICMR, India. ISBN: 978-81-910091-94

## Total biosynthesis of the tubulin-binding alkaloid colchicine

The alkaloid colchicine is a disrupter of tubulin polymerization and was approved for treatment of certain forms of arthritis and Mediterranean fever by the US FDA in 2009. However, the use of extracts of Gloriosa and Colchicum as anti-inflammatory agents has been widely reported in folklore. The activity of colchicine is presumed to reside in its tropolone ring system. Molecules carrying this unique ring system are microtubule toxins while structures where this ring system is absent are not as toxic. The substitution at the single nitrogen in the ring, especially its acetylation, is important for colchicine to retain its bioactivity. Nett and Sattely have utilized four enzymes involved in the biosynthetic pathway of colchicine in the rhizome of the flowering plant *Gloriosa superba* (Hindi: Agnishikha). The three identified enzymes were (i) CYP71DA12, which hydroxylates the methyl group of N-formyldemecolcine (m/z 400.1755) to form the hemiaminal (m/z 416.1704) which generates N-formyldeacetylcolchicine (m/z 386.1598) through the release of formaldehyde via a non-enzymatic route; (ii) alpha-beta hydrolase (ABH), which deformylates the hemiaminal intermediate to produce N-deacetylcolchicine (m/z 358.1649); and (iii) N-acetyltransferase (NAT1), which, in the presence of acetyl CoA, converts deacetylcolchicine to (-)colchicine (m/z 400.1755). These three enzymes were combined with other enzymes identified in an earlier work (doi 10.1038/s41586-020-2546-8) to reconstruct the biosynthetic pathway of colchicine in *Nicotiana benthamiana* (belonging to tobacco family). This bioengineered pathway had 17 enzymes from *G. superba* and 3 from other plants and gave rise to (-)colchicine which is the natural enantiomer. The final yield of the pharmaceutically active alkaloid in the heterologous system was 268 ng/g dry weight of plant. This work also shows that the common assumption regarding tissue-specific regulation of enzymes may need to be relooked at for production of secondary metabolites. (J. Am. Chem. Soc. (2021) 143: 19454-65)

## Red- and far-red-emitting zinc probes with minimal phototoxicity for multiplexed recording of orchestrated insulin secretion

$Zn^{2+}$ ion plays a critical role in several physiological processes like gene transcription and regulation of gene expression, biocatalysis, signal transduction and apoptosis. Hence, measurement of $Zn^{2+}$ levels in a dynamic system becomes important. Fluorescence probes have formed an important bioanalytical tool for researchers working in this area. Techniques to measure the complete spectrum of $Zn^{2+}$ ions, spanning over eight orders of magnitude, are lacking. High levels of $Zn^{2+}$ (0.01-20 mM) are encountered in secretory granules and synaptic vesicles and photostable probes to measure this range are missing. Zhang et al. started with the classic red rhodamine-based probe, RhodZin-1. The hydrophilicity of the probe was improved by addition of morpholino auxochromes (confirmed by RP-HPLC) to form PKZnR-1. Unlike sulphonation, this strategy does not alter charge on the molecule. Increased hydrophilicity reduced the interaction of the probe with cell membrane. Both phototoxicity (green light illumination) and non-specific staining of cells were reduced with the addition of morpholino moiety. Since the range of concentration of $Zn^{2+}$ to be detected is quite large (nM-mM), different groups with tunable affinity for $Zn^{2+}$ were conjugated to RhodZin-1. While the main chelating group (N,N-diacetic acid) was retained, changes were introduced in the side chain. Substitution with methoxy, ethoxy, methoxyethoxy, o-aminophenol-N,N,O-triacetic and 2-pyridylmethyl groups gave rise to PKZnR-1, PKZnR-2, PKZnR-3, PKZnR-4 and PKZnR-5, respectively. Substitution of methoxy with the bulkier ethoxy group in PKZnR-2 reduced the affinity of the probe for $Zn^{2+}$ ($K_d$ increased from 28 to 33 μM). The modular method of assembling the molecules permitted facile synthesis of tunable probes. The synthesized probes exhibited high affinity (0.19-74 μM) as well as high selectivity (over other divalent ions like $Ca^{2+}$, $Mg^{2+}$, $Fe^{2+}$, $Ni^{2+}$, etc. and peptides like GSH and glucagon) for $Zn^{2+}$. The probe with the highest affinity for $Zn^{2+}$, viz. PKZnR-5, was able to detect insulin granule exocytosis in murine and human cell clusters. More importantly, cellular fusion events could be monitored over a longer time period as the probe exhibited negligible phototoxicity. Next, the authors attempted to increase the range of these probes to the far-red region. This will permit the simultaneous detection of $Ca^{2+}$ along with $Zn^{2+}$ in cells, a commonly encountered situation. Silicon-based probes have been widely used in this space. The conventional protocols employ harsh reaction conditions with low yields. In later studies, the chelator has been coupled to the fluorophore via an amide bond. The lone pair on chelator nitrogen is not able

to quench the fluorophore efficiently, leading to faulty "turn-on" response. To overcome this, the authors developed a novel N-alkylation-based synthetic route introduced towards the end of the traditional route to give rise to the PKZnFR family. The overall yield was in milligrams. Fluorescence microscopes commonly available in the lab can be used to work with this probe as its spectral properties overlap with that of Cy5 (excitation and emission wavelengths of 654 and 676 nm, respectively). The fluorescence turn-on ratio of PKZnFRs ranged from 105-119 compared to 23 for PKZnR-1. These far-red Zn probes allowed the use of PKZnFR-3 (red, λex 647 nm) to record insulin secreted by β-cells in the presence of the mitochondrial stain, PK Mito Red (yellow, λex 561 nm) along with the nuclear stain Hoechst (blue, λex 405 nm) in islets isolated from transgenic mice expressing GCaMP6f (green, λex 488 nm). The authors hope that the designed probes and their orthogonal use will meet the needs of "4D physiology in big data era". (Angew. Chem. Int. Ed. Engl. (2021) 60: 25846-55)

## Impact of simulated intestinal fluids on dissolution, solution chemistry, and membrane transport of amorphous multidrug formulations

As per the definition provided by Central Drugs Standard Control Organization (CDSCO), "Fixed Dose Combinations refer to products containing one or more active ingredients used for a particular indication(s)." Fixed dose combinations (FDCs) have found enormous importance in clinical practice, especially in the treatment of chronic diseases. FDCs improve patient compliance and reduce the economic burden on the healthcare system. However, the effect of different drugs on properties of each other, e.g. miscibility, solubility, active concentration, extent of ionization, etc., has not been investigated in a systematic manner. The use of amorphous solid dispersions may be adopted to improve solubility of poorly water-soluble drugs. For example, supersaturation/solubility of components of amorphous FDCs in a biologically relevant medium has not been studied rigorously. El Sayed et al. studied two combinations, the first one for anti-retroviral therapy, which combines atazanavir and ritonavir, and the second one for anti-hypertension therapy, which combines felodipine and indapamide. The crystallinity of all four drugs was confirmed by DSC and PXRD. The resulting two-drug formulations were amorphous. Conversion of amorphous form was seen to increase solubility in buffer and fasted state simulated intestinal fluid (FaSSIF). In a mixture of atazanavir and ritonavir using PVP as the matrix, the supersaturation of atazanavir and ritonavir in FaSSIF decreased by 50% and 30%, respectively, as compared to the single drug. Interestingly, in the second mixture, only marginal decrease in solubility was seen in case of felodipine in a mixture when compared with the free drug while indapamide showed significant decrease in dissolution at all time points of incubation when present in 1:1 ratio with felodipine. Mass-transport profiles showed similarity in transport pattern of drugs across membrane (regenerated cellulose) in either group, either when considered alone or in combination. No difference was observed in buffer or FaSSIF. Membrane transport was seen to decrease by ~40-50% for atazanavir and ritonavir when present in combination as compared to the drug alone. A similar reduction was seen in case of indapamide. However, no difference in flux was seen in case of felodipine, either alone or in combination. The flux ratio was ~1 in all cases suggesting that the complex media (FaSSIF) had no measurable effect on transport across membrane although the solubility ratio was tilted positively towards FaSSIF. This resulted in overestimation of the amount of drug actually being transported across the membrane and may account for the observed results. Amorphization led to the formation of a miscible system between uncharged atazanavir and ritonavir. Reduction in maximum achievable concentration in the multidrug combination was proportional to the mole fraction of each drug. The difference in dissolution of ritonavir component of the multi-drug formulation in buffer and FaSSIF was attributed to its differential solubility in the two solvents. The authors have proposed a formula by taking into account the amorphous solubility of the drug in buffer and the micellar contribution (difference in the observed solubility of amorphized drug in buffer and FaSSIF) to predict the solubility of the drug in a two-drug formulation. They project that the same formula can be extended to predict the solubility of individual drugs in multi-drug formulations in other surfactant-based biomimetic systems which exhibit solution behaviour similar to atazanavir/ritonavir. The equation could be used successfully to predict and experimentally confirm the dissolution behaviour of indapamide in a two-drug formulation with felodipine. The solubility behaviour of felodipine, however, could be correctly predicted only in the presence of lower concentrations of indapamide and not at higher concentrations. (Mol. Pharmaceutics (2021) 18: 4079-89)

## Caffeine as a viscosity reducer for highly concentrated monoclonal antibody solutions

Due to their remarkable target specificity, monoclonal antibodies account for a major fraction of applications of biopharmaceuticals. High concentration, with

resultant high viscosity, is a barrier to the use of monoclonal antibodies for manufacturing and therapeutic purposes. Intravenous infusion requires trained health practitioner which adds to the cost of therapy. Traditionally employed excipients like arginine may increase solution viscosity in a few cases while some other viscosity-lowering agents are yet to receive regulatory approval. Zeng et al. have investigated the use of the alkaloid, caffeine, which is acceptable to regulatory authorities, as a viscosity-lowering agent, with the aim of developing a sub-cutaneous formulation. The authors have compared the viscosities of two clinically important monoclonal antibodies, viz. infliximab (marketed as Remicade®) and ipilimumab (marketed as Yervoy®), by formulating them in 75 mM caffeine and in 100 mM NaCl and 100 mM arginine (ArgHCl). The procured antibodies were buffer exchanged and formulated in 20 mM phosphate-acetate buffer, pH 6.0 or 20 mM histidine buffer, pH 5.5, for infliximab and ipilimumab, respectively. Addition of NaCl or arginine led to no change or slight increase in viscosity of both antibody solutions. However, addition of caffeine led to ~77% reduction in viscosity of infliximab (150 mg/ml). A similar order of reduction was seen when ipilimumab (150 mg/ml) was reconstituted in phosphate-buffered saline (PBS). When formulated in 20 mM histidine buffer, pH 5.5, addition of caffeine led to reduction in viscosity of the antibody from 29 cP to 21 cP (28% reduction). Addition of caffeine lowered attractive protein-protein interaction in infliximab by 66%. No effect of caffeine was seen in case of ipilimumab. Biolayer interferometry showed weak binding between the two antibodies and caffeine. As Remicade® is sold as a lyophilized powder, the effect of caffeine on the lyophilized antibody was studied. Different concentrations of caffeine were used for lyophilization and no difference in reconstitution time was seen when compared with the control (no caffeine). Depending on the concentration of caffeine in the formulation, 60-80% reduction in viscosity of the reconstituted formulations was reported. The viscosity-lowering ability of caffeine was retained when the formulations were incubated at 4°C for 37 weeks. SEC-HPLC revealed no difference in the monomer composition in any formulation immediately upon reconstitution or after incubation for 12 weeks at 40°C between control and caffeine-containing formulation. Cation exchange chromatography too showed no difference in charge variants in the presence of caffeine. Yervoy® is marketed as a solution formulation. In the presence of caffeine, no significant difference in monomer content of ipilimumab (200 mg/ml) was seen as compared to control when stored at 4°C/16 weeks or 40°C/6 weeks. The degradation pattern of the antibody also remained unchanged upon storage. TNF-? neutralization assay and CTLA-4-based ELISA showed that caffeine had no effect on the functionalities of infliximab and ipilimumab, respectively, in vitro. As caffeine has multiple advantages in terms of regulatory acceptability and favourable pharmacokinetic profile, the use of caffeine as a viscosity-lowering agent in high dose formulations may be explored further. (J. Pharm. Anal. (2021) 110: 3594-604)

## First COVID-19 DNA vaccine approved, others in hot pursuit

The Emergency Use Authorization of ZyCoV-D by the Drugs Controller General of India (DCGI) has been hailed as "a milestone for a nucleic acid technology". The DNA-based vaccine (encoding spike protein and carrying IgE secretion signal) against SARS CoV-2 has been developed by Zydus Cadila, an Indian pharmaceutical company. It uses a needle-free jet injector (PharmaJet® Tropis®) for intradermal delivery of the vaccine, which is classified as 'safe' by WHO. It has been proposed that an alternate delivery route may lower the cost of the vaccine. DNA vaccines offer several advantages like ease of processing, longer period of response and enhanced stability. The major concern in their use has been regarding the possibility to get integrated into genomic DNA although no evidence has been offered in the literature to this effect. The article highlights the different requirements and cellular targets of mRNA- and DNA-based vaccines. This is clear from the dose of ZyCoV-D which is reported to be 2 mg of plasmid DNA each for three doses as compared to two doses of 30 µg each for BNT162b2, an mRNA vaccine. Peer-reviewed clinical trial data for ZyCoV-D has not been made available yet. The article traces the history and early failures of DNA-based vaccines which paved the way for mRNA to be developed as a vaccine platform. The article notes that since ZyCoV-D is cloned in a commercially available plasmid and follows a generic manufacturing protocol, its production can be extended to third parties and hence availability of the vaccine will not be a factor of concern. The vaccine exhibited 67% inhibitory efficacy in case of symptomatic infection and was fully effective in averting moderate disease. These numbers, and their comparison with other approved vaccines, may not mirror their usefulness in a clinical setting as the emergence of new strains and post-hoc data analysis force the revision of available data with vaccines which have already been approved. (Nat. Biotechnol. (2021) 39: 1479-1485)

# NIPER News

## Gender Sensitivity Lecture

On 01.10.2021, there was a lecture organized on the title "Are we gender sensitive? Let us introspect!" by Prof. Rajesh Gill, Dept. of Sociology, Punjab University.

## Rashtriya Ekta Diwas Celebrations

To commemorate the birth anniversary of Sardar Vallabh Bhai Patel on 31st October, 2021 as National Unity Day (Rashtriya Ekta Diwas) in convergence with the activities of Azadi Ka Amrit Mahotsav, the institute organized Events from 24th to 31st October, 2021.

Prof. VK Kapoor delivered a lecture on "Scientific Development of India in the last 75 Years, Particularly with Reference to Pharma". There was a lecture by Prof. Rajesh Kochhar on "What is Nation?: A global perspective".

The Unity March was organized on 25th October 2021 inside NIPER Campus. The staff, students and outsourced staff Marched shoulder to shoulder raising slogans of "National Unity Zindabad", "salute to the Martyrs of freedom struggle". There was Cultural Evening organized on 28.10.2021 at NIPER Convention Centre. The Patriotic Play was performed by Alankar Theatre Group. The Play depicted the life struggle of many unsung heroes of Indian Freedom Movement. On 29.10.21, a Unity Rally organized in City around NIPER.

## SERB 'Karyashala'

The institute organized an online High-End Workshop 'KARYASHALA' Funded by Science and Engineering Research Board (SERB) under the "ACCELERATE VIGYAN SCHEME" on 'FLOW CYTOMETRY CELL DEATH AND DRUG DISCOVERY' from 17th-23rd October, 2021. This Karyashala was organized by Dr. Sushma Singh, Associate Professor, Department of Biotechnology, NIPER, S.A.S. Nagar. The applications were invited from students all over India. Fifteen doctoral students and ten post-graduation students from various states and national institutes were selected as per the guidelines provided by SERB. The workshop involved seven days of rigorous training on the basics of Flow cytometry and its applications. This event was organized in collaboration with Flow cytometry solutions and Thermofisher. It included various sessions on experiments related to understanding cell death and drug discovery. Special emphasis was given to train the participants in interpretation and analysis of the flow cytometry data. In the later part of the event, various eminent scientists from all over India shared their expertise and experience of using this technique in drug discovery.

## NIPER Convocation

The institute held its 12th convocation on 25th November, 2021. Total 260 students (250 Masters and 10 Ph.D) received their degrees.

A mini Marathon was organized in the Campus on 04.12.2021 through ISPOR NIPER Student Chapter to educate the participants about the potential benefits of physical activity on health. On 07.12.2021, an awareness campaign on expired medicines was organized not only provide a safe, convenient and responsible means of disposing of medications but also to educate the general public about potential risks of keeping those medications in home.

## Skill Vigyan Training Programme

NIPER SAS Nagar is organizing DBT sponsored Skill vigyan training programme on module "Quality Management System In charge" from 27.12.2021 to 24.03.2022. The institute is striving to align skill development efforts to the Nation's skill India mission of GOI by continuously organizing such training programmes. This training programme will empower the participants to upgrade their skills along with providing them better job opportunities in future.


12th Convocation of NIPER S.A.S. Nagar held on 25th October, 2021


Unity March across Mohali city by NIPER employees and students


**Inauguration ceremony of "Azadi Ka Amrit Mahotsav"**


**Hon'ble Dr. Mansukh Mandaviya, Minister of Health and Family Welfare, Minister of Chemicals and Fertilizers and Ms. S. Aparna, The Secretary, Department of Pharmaceuticals addressed students of all NIPERs during "NIPER Week" as a part of "Azadi Ka Amrit Mahotsav"**


**Rashtriya Ekta Diwas Celebration in NIPER, S.A.S. Nagar**