Contents

Title of the Project: ***Design of Combination Drugs for the Treatment of COVID-19***		
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Outcome:

Objective1: ***Combination drug leads for COVID-19***

Objective2: ***Validation of the efficient approach for COVID-19 combination drug identification*****: The three approaches to be tried are a) Our methylene linking & in silico docking followed by MUNANA assay for COVID 19; b) Identifying second binding site on neuraminidase for the 2nd combination drug to bind to COVID-19; c) One drug each for neuraminidase & hemagglutinin: The two peripheral viral proteins, which help virus to enter & replicate host human cell**

Objective3: ***Comprehensive process for combination drug identification for any bacteria, virus, drug resistant strains: A platform software*****, which does batch wise combinatorial screening using clinically approved drugs & in vitro validation of obtained results.**

Project Outcomes (Timeline based):

0-4 Months: COVID-19 combination drug identification for Clinicians for trials

5-8 Months: Combinatorial screening of different drug molecules as combination drugs for neuraminidase & hemagglutinin for COVID19

9-12 Months: Combination Drugs to treat COVID-19

3. Detailed Proposal with experimental details, objectives & milestones

1. Preamble

COVID-19 is an example of what humanity is going to face and the new medicines or drugs are hard to come by. This single drug treating a single disease is not going to sustain due to rapid mutations in virus genes and the newer virus penetration from wild animals to human body. The situation of COVID-19 or in future new diseases is going to prevail and we need a sustainable solution to rapidly identify potential combination of drugs, which can work in shortest span of time.

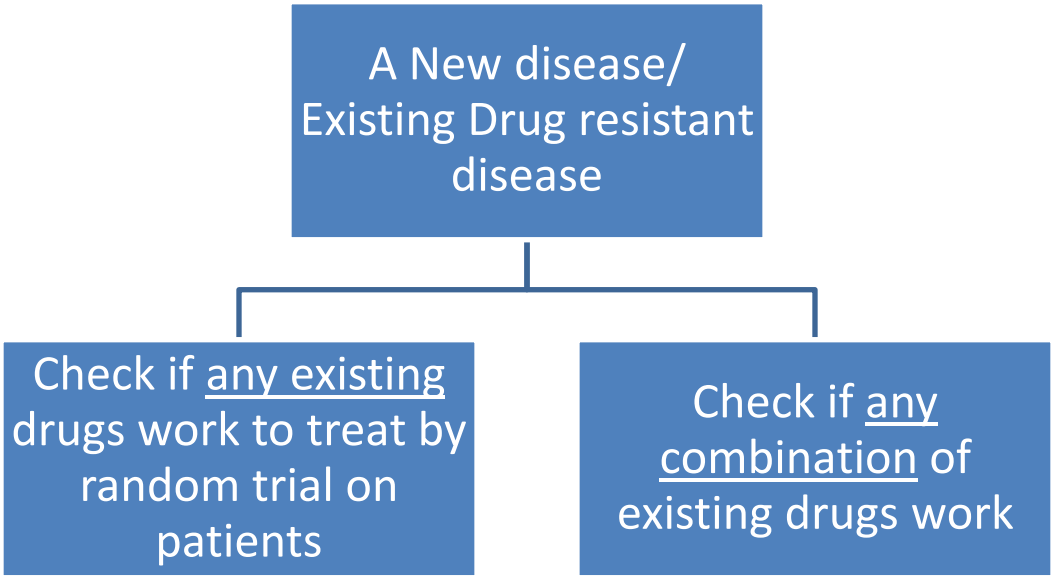

Figure 1: Current medical practice of testing existing drugs for any new disease or drug resistant infection by clinicians. Same approach was followed for Zika, Ebola, Swine flu, MDR tuberculosis, XDR tuberculosis and humanity is lucky to have had a hit in these random trials. But, for COVID-19, no single drug or combination drug has worked till now.

2. Executive Summary

Problem 1: When newer drug molecules are developed, the medicinal chemists follow a rule called Lipinski rule of five wherein it indirectly says smaller the molecule, better is the Pharmaco- safety of it because the secondary metabolites will be simpler chemicals. In current drug development scenario, most molecules do not obey Lipinski rule of five and have poor pharmaco- safety profile and hence newer drugs are difficult to be discovered. Add to this that last 2 decades have seen only few antibiotics

and antivirals and that too for which resistance development by virus/ bacteria is already reported. Add to this, the existing antibiotics/ antivirals are not effective against newer diseases. COVID-19 is an example of it wherein none of the known drugs are able to act on it.

Kerala awaits ICMR nod on use of Cuban drug

Thiruvananthapuram: The Kerala government, which is awaiting permission from ICMR and Drug Controller General of India for the trial of Cuban drug interferon alpha, only intends to try it in extreme cases and it may not be a ready-to-go drug for Covid-19 in other categories.

State planning board member Dr B Ekbal, who pitched the idea of using this drug in emergency cases, said that it is not a wonder drug and had been in use. "We plan to conduct a trial for which permission is awaited. This drug was used for treating Hepatitis C. We can do a trial and find if it is useful in severe cases," he said. TNN

Assam doc died after taking malaria drug?

Prabin.Kalita@timesgroup.com

Guwahati: A senior anaesthetist at a private hospital in Guwahati who was on hydroxychloroquine to protect Covid-19 infection — as are many other doctors elsewhere in the country — died of a heart attack here on Sunday afternoon after posting a message to colleagues on a WhatsApp group saying he thought he was having "some problems" after taking the medicine.

There was, however, no way of knowing immediately whether his death was a direct consequence of using the drug. "Without a postmortem one cannot explain Barman's death," Guwahati-based internal medicine specialist Swarup Jyoti Saikia said, adding that the death did, however, raise "a red flag". Doctors said they would probe the matter thoroughly.

Utpaljit Barman (44), who headed the anaesthetics team at Pratiksha Hospital, had sent a message to colleagues in a WhatsApp group about half an hour after suffering the heart attack at home saying, "HCQS (hydroxychloroquine) is not good as prophylaxis (action taken to prevent disease). Lots of issues. I think I am having some problems after I took it."

The anti-malaria drug is commonly used to treat rheumatoid arthritis. "This drug is now also prescribed for some cases of uncontrolled diabetes," he added.

Full report on www.toi.in

Tamiflu stars in first recoveries

Karnataka's first six Covid-19 patients have all been discharged after testing negative twice in 24 hours, reports **Sunitha Rao R.** Doctors said Tamiflu, the antiviral drug used to treat Influenza A H1N1, helped them recover. Besides Tamiflu, paracetamol and supportive drugs were used. Most patients are exhibiting mild to moderate symptoms, officials said. **P 4**

TB vaccine investigated as a weapon against coronavirus

F1 team, varsity make new breathing aid

Migrants 'sanitised' with bleach, probe on

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Bareilly: Several batches of migrant workers, including women and children, who arrived in UP from Delhi/NCR in buses over the weekend were made to squat on a road in Bareilly district and sprayed with sodium hypochlorite — a bleaching agent which authorities later admitted was "unfit for use on humans" — in a bid to sanitise them.

After the incident on Sunday attracted widespread criticism, joint secretary (health ministry), Lav Agarwal, blamed it on

UP SHOCKER

the "over zealousness of officials who acted out of fear". The secretary clarified that this was not part of any protocol and was not required.

The Bareilly district administration has now set up a team to investigate the matter. District magistrate Nithish Kumar said treatment would be provided to anybody adversely affected by spraying of sodium hypochlorite. The DM also attributed the incident to "overcautious" personnel. "They should not have done this, but fortunately no one was harmed," he told **TOI**.

Mayor Umesh Gautam admitted "negligence on part of the municipal corporation" officials.

Full report: www.toi.in

(With inputs from Maria Khan)

Figure 2: The current clinical practice of usage of drugs on random basis is depicted in 31st March 2020, Times of India: different approaches tried by clinicians in a single day with minimal logic.

Problem 2: Chemical drug development itself is currently looking for ways to use combination drugs. Last 2 decades have seen five epidemics and all of them needed combination drugs and for COVID-19, no single drug is effective, clearly underlining the need for combination drugs. Even if we count two molecule combination drugs as a possibility, we get about 80,000 drug combinations for currently approved 400 drugs, which is literally impossible to test clinically for each new disease discovered. THE WORLD NEEDS A SIMPLER WAY TO SCREEN POSSIBLE DRUG COMBINATIONS TO USE AGAINST A NEWER DISEASE OR RESISTANT DISEASE STRAIN. AS OF NOW THE WORLD HAS NO METHODOLOGY FOR THE SAME AND OUR RESEARCH GROUP HAS FIGURED OUT A WAY TO SCREEN THOUSANDS OF COMBINATION DRUGS. As of now, the COVID-19 pandemics treatment is possible with combination drugs and the said combinations already tried are hydroxy chloroquine- azithromycin; oseltamivir-azithromycin; paracetamol- oseltamivir; ibrufen-zanamivir-oseltamivir; interferon-alpha-oseltamivir etc. None of the combination drugs till now have yield concrete solution because each of them is random combinations based on patient symptoms and not based on scientific logic of combating the COVID-19 virus.

Solution Proposed: We have a developed a multi ligand docking approach, which was done for swine flu (H1N1) as target in the year 2012-2013. This approach relies on principal that a) protein has multiple ligand binding sites b) Some of the protein like the one in COVID-19, the neuraminidase, has larger protein ligand binding site, which can be blocked by a combination drug and not a single drug. When these two aspects are taken together, they augment for combination drug approach, which is very simple to get approved and can be deployed easily because each of the Indian drugs are already Indian FDA/ ICMR/ Drug regulators approved. The narrowing down from few thousand to few million possible combinations to 2-3 types of combination drugs, will help the clinicians rapidly evolve a solution. This approach is practical to use and probably only hope of surviving current COVID-19 and also future epidemic survival. In this project, we will screen the drug combinations with both of our multi ligand docking approach and second drug binding site approach. Kindly note that current docking tools allow only one ligand docking with one protein and this combination drugs cannot be screened, in silico. The in silico results are rapidly verified by in vitro screening to arrive at combination drugs with antiviral activity. The uniqueness of this approach is that the individual drugs may or may not have any antiviral activity, but as combination, they actually demonstrate activity and hence the combination drugs are the way forward with rapid verification of in silico results with in vitro screening. The in vitro verification

is significant enough to make recommendation for clinicians to aim for a clinical study because each of these drugs is in clinical use for humans. Our approach is a new way and it will simplify the clinician's choice to give combination drugs and probably the only hope of identifying rapid treatment for COVID-19 and for future endemics/ pandemics.

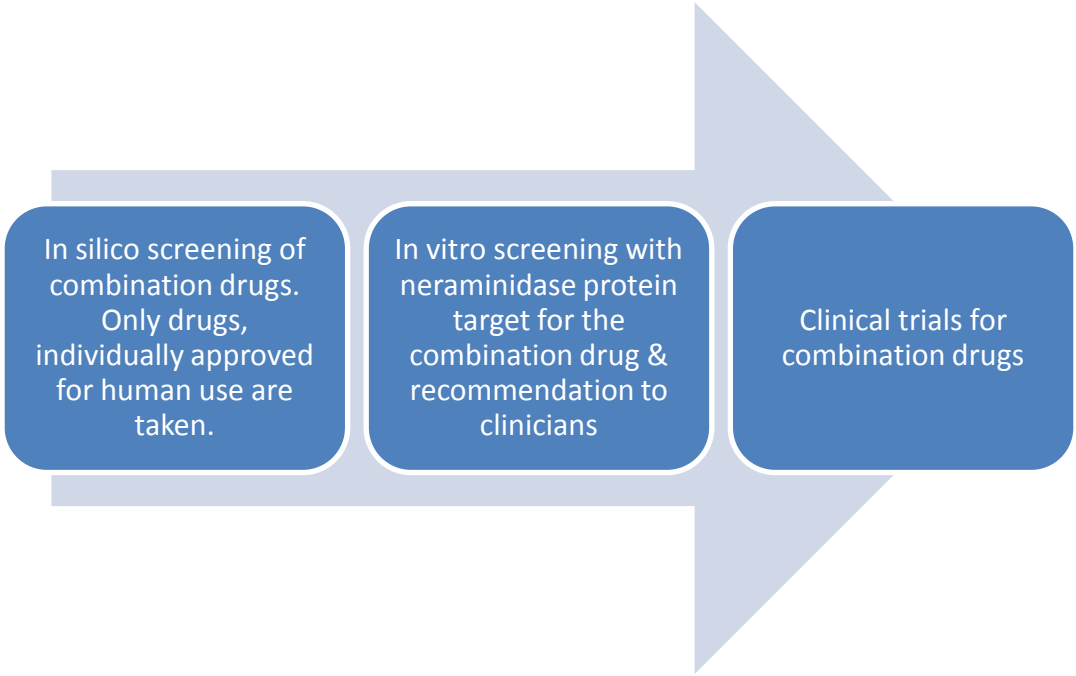

Figure 3: This is our proposed COVID-19 combination drug methodology, which can have a cure in less than 3 months: The drugs which are already approved for human use are tried as combination drugs in silico and from few thousand drug combinations possible, the ones with maximum possible interaction are recommended for in vitro neuraminidase fluorogenic enzymatic screening. The reconfirmation in enzymatic activity will allow direct recommendation for clinical trials because these drugs individually are already recommended for patient use individually. The proposed methodology is quickest possible path for identifying combination drugs for COVID-19. Neraminidase enzyme target is a universal influenza target for both monoclonal antibody based (MAb) drugs and for chemical drugs.

3. Title of the project: Rapid Re purposing of existing drugs for any new epidemic/ pandemic including COVID-19- Combination drugs for clinical therapy

4. Introduction/ Background: Whenever a pandemic or epidemic occurs, single active ingredient containing western drugs will struggle because of resistance development. Multiple drugs targeting single disease target is probably the only way forward. This was proven with tuberculosis- 3 drugs, HIV-

3 drugs as combination. The world is lucky to have for H1N1, oseltamivir & zanamivir as re purposed drugs but the same did not work for COVID-19. There are over 80,000 possible TWO-DRUG combination of western drugs and if THREE Drug combination is taken, then the number is close 10 million combinations. Literally, no one can try this many combinations for a single disease. Added to this problem is the drug resistance by virus and bacteria. THIS PROJECT IS TO CONNECT COVID-19 DISEASE PROTEIN TARGETS WITH COMBINATION OF Chemical drugs and suggest possible drug combinations for clinicians to arrive at a therapy, which saves lives. The PI of this proposal has worked on combination drugs, developed multi ligand approaches for Swine flu (H1N1) control and in general has years of high impact research experience on identifying multiple drug targets on proteins & designing drugs for the same.

SNo	**Number of existing drugs as combination**	**Total combinations possible for clinicians need to try**
1	Single drug	400
2	Two drug combination	80,000
3	Three drug combination	~10 million
4	Four drug combinations	1 billion

Table 1: Stastical reality of different combinations of drugs possible with currently approved 400 odd clinical drugs present in market with a SINGLE protein target. Only in silico combinatorial screening can handle this many combinations and it is high time clinicians' hands are emboldened.

5. **Premises/ rationale on which the present proposal is based and justification for taking up the project:**

Any influenza (corona) virus surface has typically two protein targets namely hemagglutinin, and neuraminidase. The later viral protein binds to sialic acid of cells to make its entry into host cell and multiplies. The neuraminidase protein of virus has binding site, known to possess large cavity in which more than one ligand can bind and hence the combination drugs are typically a strategy. The COVID-19

is like SARS, H1N1 where in the neuraminidase is not mutated and as such is an incredible target for prophylaxic approach for virus prevention.

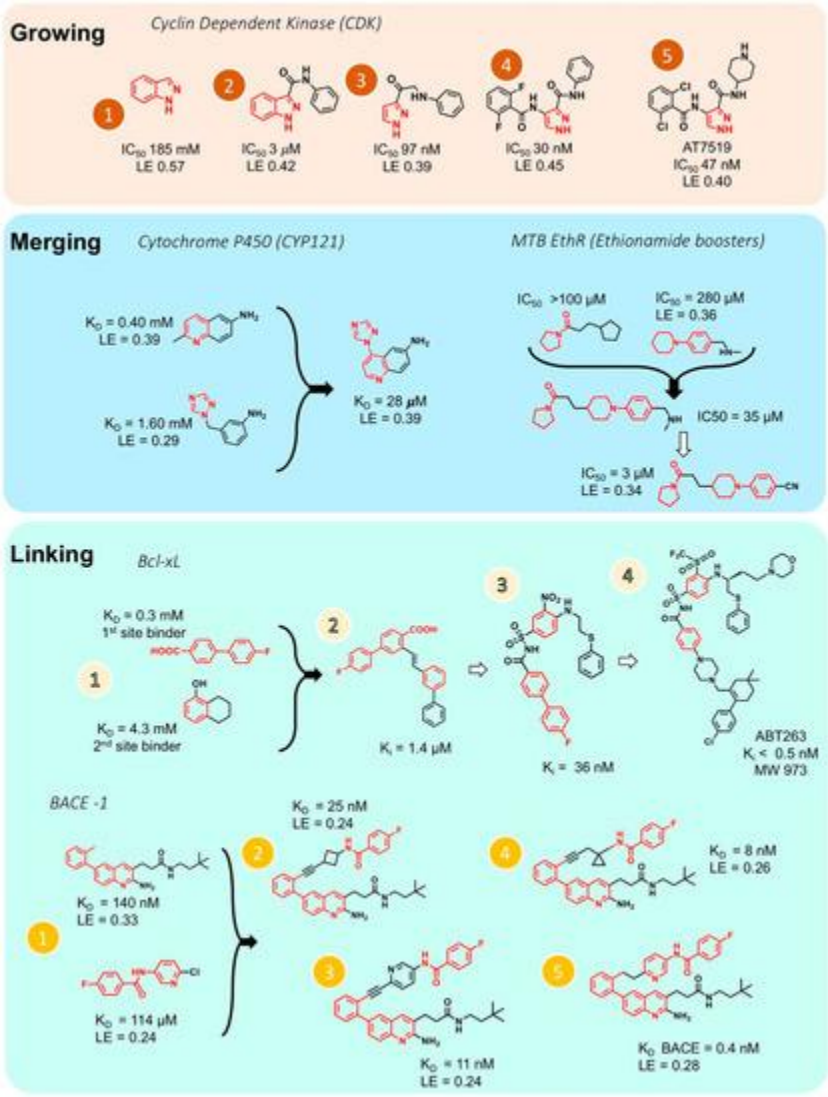

Figure 3: Different hit-to-lead optimization strategies a) fragment growing, b) merging and c) linking approach). Clear demonstration of reason for efficiency of combination of active molecules is the way forward. Unfortunately, it takes nowadays 12-15 years to a get single drug approved with an estimate of US$1.5 billion ("NMR-Fragment Based Virtual Screening: A Brief Overview" Molecules 2018, 23(2), 233). It can also be noticed that the increase in molecular weight of molecules is clearly suggesting violation of Lipinski rule of Five, a medicinal chemist's preliminary tool to evaluate suitability of chemical molecule as a drug.

The world which believes in vaccine to protect against infectious diseases is coming in terms with idea of prophylaxic molecules, especially for new diseases for which vaccine development & approval takes time. The combination drugs are now the new hope for society considering the new drug development is incredibly low due to most simple chemical molecules under molecular weight 300 are covered in some or other patent and the typical drug lead to medicine development time of 10-12 years.

6. Objectives of the Project

Objective1: Combination drug leads for COVID-19

Methodology/Experimental Design To Accomplish The Stated Objective:

Step 1: Use of our methyl linked multi drug docking to identify the docking energies and specific amino acid interaction to narrow down the combinations for protein target of COVID-19

Step 2: Ideally combination drugs of 2 is preferred over 3 but most successful combination drugs especially for virus have been of 3 and combination drugs will be explored in both 2 or 3 combinations.

Step 3: Narrow down the combinations from multiple drugs binding to multiple protein targets in virus to multiple drugs targeting same viral protein and ascertain the clinical combination drugs by allotting scores

step 4: Inform the same to clinicians for broader debate and implementation

Alternate Strategies: As mentioned, we can always take a protein already docked with a ligand and treat as a new protein target to identify peripheral protein binding sites on the same protein. multiple ligands binding to protein seems to translate to significantly improved binding constants. This was proven by us in our Cytochrome P450cam paper and also our groups DHPR NMR based biligand strategy paper.

Process Indicators for Measuring Success: The shortlisted COVID-19 combination drugs will be complete within first six months of the project. The three approaches of identifying ligands from existing drugs for a Viral Neuramindase protein b Drug combination to bind to hemgglutinin & neuraminidase from the combination drugs c Thirdly the drug combination binds to the oseltamivir/ hydroxychloroquin docked neuramindase. This result will quickly give a combination drug for COVID-19 and pave way for a

lifesaving medication. Hope of a single drug being medicine for COVID-19 looks dim and this combination drug approach is what can take it forward.

Objective2: Validation of the efficient approach for COVID-19 drug combination identification

Methodology/Experimental Design

To Accomplish The Stated Objective, The three approaches are a) Methylene group connected drug combinations docked with potential protein target using DockThor etc. b) Using the drug bound protein as a means and tricking the DockThor to allow another ligand docking to identify secondary binding site on the same protein which is known to improve combination drug efficacy by many orders of magnitude. C) Identify multiple viral protein targets and identify the drug combinations which can work. This third approach can involve known drugs for that potential protein targets or known drugs for the docking with unknown affinity for a drug molecule

Alternate Strategies: These three strategies comprehensively cover the possibilities and we will obtain leads.

Process Indicators for Measuring Success: This is more for refining the above objective 1 established leads for the COVID-19. this extensive study will build a process for creating a methodology

Objective3: Comprehensive process for combination drug identification for any bacteria, virus, drug resistant strains & targets.

Methodology/Experimental Design To Accomplish The Stated Objective: There are existing combination drugs known for HIV, Tuberculosis, Zika, Swine flu, Ebola, malaria etc and could be used a basis set to validate and refine the above developed approach. as there is no method for determining combination drugs and currently it is mostly by trial and error and luck, this approach needs standardization clinicians to have shortlisted combination drugs to go forward by data of weighted average of toxicity, dosage, combination interference etc.

Alternate Strategies: At this stage we have to look for ways to have clinical data and combination drug data validated with a regression coefficient for future combination drug discovery as well as a rapid standardized process for newer protein/ pathogen targets.

Process Indicators for Measuring Success: The regression coefficient of actual clinical drug combinations versus our *in silico* drug combination suggestions will make it a path forward. The existing combination

drugs may look minimal but will take us definitely towards building a universal model and the need for same is ever needed by the drug discovery community.

7. Current status (give brief details of present status of developments- R&D and industry -nationally and internationally including IPR status)

R&D: The new drug development has slowed down significantly in recent years due to longer gestation period and significant mandate of regulatory agencies for new drugs. The typical validity of a patent of 20 years looks too less from patent filing because of the 12-15 years' time taking for discovery to product launch. There is an estimate that it costs over US$1 billion to obtain a discovery stage molecule to market ready product crossing through the preclinical, clinical and regulatory approvals. This expensive and endless process coupled with perception by developed world that investment in new antibiotics and new antivirals only serve the developing world and the investments in same are strongly discouraged by Venture Capitalists (VC). The hope of having newer drug molecules for newer diseases is no more and the need of the hour is the combination drugs. Combination drugs are typically deployed for treatment of patients with tuberculosis, HIV, H1N1, pneumonia, bronchitis etc. The interesting aspect is that combination drugs are basically two or more drugs which are already in use (probably from tens of years) and when given as combination, the bacteria/ virus takes time to develop resistance and also they act together on multiple pathogen targets thereby slowing down spread of infection and buying the time for body's natural immune response to kick in. R&D in medicinal chemistry for single molecules is so crowded that any simple molecule theoretically possible with molecular weight of molecule less than 500 is hard to come by with crowding of patents.

Industry: Industry does not like re purposing the existing drugs because, combination drugs can be approved less than a week's time. For example, for COVID-19 paracetamol in combination with chloroquine could have some beneficial affect and in less than 3 days ICMR has approved the same for health workers in India. The beauty is that the each of the combination drugs are already in clinical use and at some level, clinicians are even aware the side effects, etc and hence they are a nightmare for the industry. When the swine flu outbreak was there, the oseltamivir, zanamivir combination was recommended and now H1N1 is more or less like regular flu in India. The combination drugs have also the advantage of slow drug resistant from the pathogen because each of them may be acting at different targets or in a peculiar way in the same binding site. The industry reluctance for newer

antibiotics and anti virals because of the perception that these are needed for only for third world countries and they have given significant importance for drug development for lifestyle diseases like cancer, ulcers etc. The world is at least 15 years from seeing any useful single molecule drugs as proper antibiotics and anti virals and hence combination drugs seems to be the way wherein the drug approval could take less than a week and, in a way, industry needs this time given by the combination drugs. Certain markets like USA are refusing to allow combination therapy due to strong industry lobby but Indian regulators are apt for it and are the way forward.

8. Novelty of the proposal

The approach can be viewed in two paths for COVID-19: Path 1: It is a virus and its neuraminidase protein with ligand bound like oseltamivir, zanamivir or any other retroviral drug structure is known in PDB, we will dock another ligand the FDA approved drug molecule to identify the secondary binding This is similar to our paper on P450cam having secondary binding site on periphery/ surface of the protein which binds and probably interacts with the primary ligand. The IC50/ Kd values may be in the order of few micromolar but the cumulative effect of protein- ligand interactions will be exponential in nature. For example in our DHPR approach, we took micromolar affinity ligands and bonded the two with a linker when the resultant ligand has shown sub nanomolar affinity and it is well proven by our research group in bi ligand strategy including experimentally. This DHPR biligand approach has resulted in US66 million dollar license of the resultant drug and Dr. Pullela worked on this project when working as postdoc. The subset if the P450cam approach wherein again by NMR it is proven that the two ligands need not be physically bonded and the van der waal / hydrogen/ hydrophobic interaction between two ligands is good enough to increase the overall antagonist effect on the protein target.

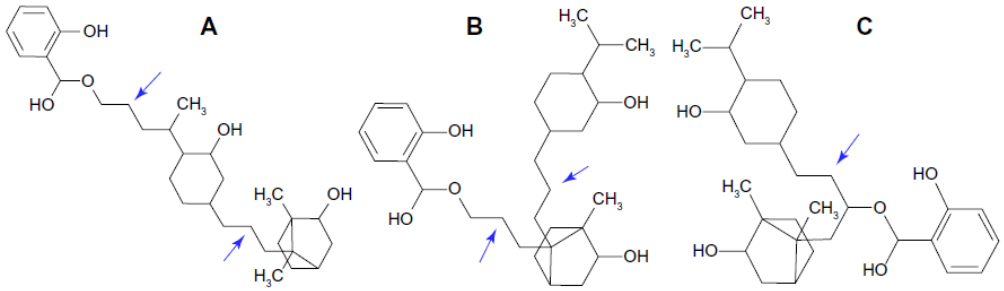

Figure 5 Chemical structure of combination ligands. Methyl salicylate-menthol-camphor (**A**), methyl salicylate-camphor-menthol (**B**), and camphor-methyl salicylate-menthol (**C**). The arrows show the methylene bridges artificially created to enable the possibility of docking the combination ligand with the enzyme. Note the drop in docking energy of **B** (–11.18 kcal/mol) when menthol and camphor position is interchanged from **A** (–12.68).

Figure 4: OUR Group Work: A land mark publication showing that a combination of camphor, methyl salicylate & menthol is equivalent to sialic acid, & oseltamivir drug. A complex disease like Swine flu is now non-existent because the typical cold vaporizer/ inhaler is now the first line of defense. (Narayanan, Manoj M, PK Pullela et al "Design of multiligand inhibitors for the swine flu H1N1 neuraminidase binding site" Advances and applications in bioinformatics and chemistry 6, 47-53 (2013).

Path 2: In this approach, we use a linker usually methyl group to connect two combination drugs and check the docking energy with protein structure from PDB. please note that as such we cannot do multi ligand docking on a protein and above approach is one way and second is this approach pioneered by our group wherein we chemically connect the prospective drugs to bring in two potential drugs to interact with binding site. The amino acid specific interactions clearly undermine the potential of a drug combination to work for a disease target protein.

These two approaches are the probably few ways to narrow down the few lakh combinations to few combinations. The bioinformatics is now powered by large clusters of servers and with a simple infrastructure of few lakhs and in few weeks' time, we can filter through the possible combinations to find potential combination drugs to treat newer infectious diseases like COVID-19 and also for existing infectious having drug resistance for commonly used antibiotics and retrovirals. The experimental design for COVID -19 combination drug results will be delivered with in first 6 months: The Protein Data Bank crystal structure of the neuraminidase in H5N1 bound to oseltamivir will used as a reference and a

model built for COVID-19 neuraminidase will be used for docking studies. DockThor10 The Scripps Research Institute, La Jolla, CA, USA will be used for protein-ligand docking studies. Drug chemical structures will be downloaded from Pubchem and similar databases and verified or drawn using ACD Chemsketch Advanced Chemistry Development, Inc., Toronto, ON, Canada. Protein electrostatic potential will be calculated using the Adaptive Poisson Boltzmann Solver ABPS with DockThorTools release 1.5.4. Molecular graphics images will be produced using the UCSF Chimera package from the Computer Graphics Laboratory, University of California, San Francisco, CA, USA. The database of individual drug combinations with neuraminidase protein target will be populated in the server and the results are interpreted based on docking energy and individual amino acid interactions to arrive at the potential drug combinations. a dynamic scoring mechanism will be arrived by utilizing weightage for different parameters and some existing drug combinations will be used a basis set to validate the *in silico* analysis method.

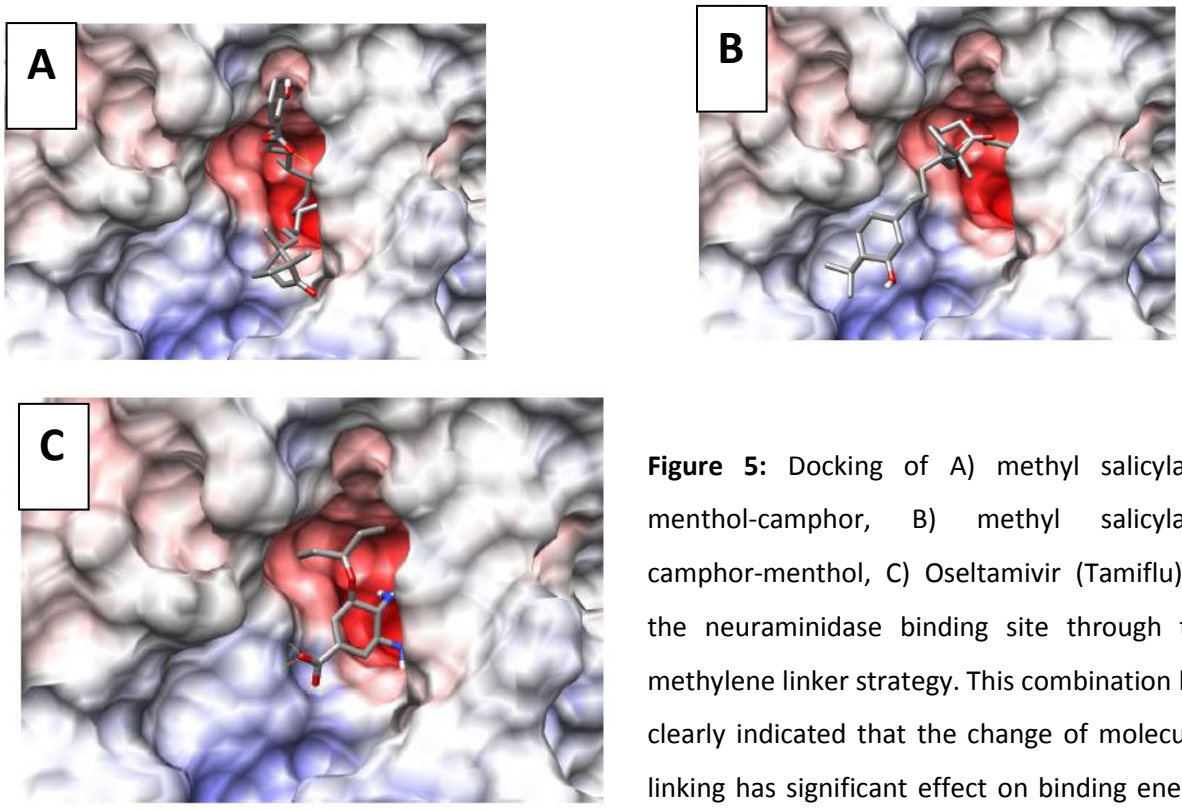

Figure 5: Docking of A) methyl salicylate-menthol-camphor, B) methyl salicylate-camphor-menthol, C) Oseltamivir (Tamiflu) in the neuraminidase binding site through the methylene linker strategy. This combination has clearly indicated that the change of molecules linking has significant effect on binding energy as well as amino acid interaction. This kind of reversible simultaneous binding is one of the mechanisms for combination drug binding and could suggest potential combinations for clinicians to use.

Always in silico methods are viewed with perception that they are not accurate but here the scenario is with already approved drug molecules and as such it is the only practical way to arrive at combination drugs for clinicians to try and validate. The new drug discovery is going 'no where' and this simple approach can narrow down lakhs of combination drugs possible few which can clinically tested with possibility of faster success in saving lives during endemics and pandemics. The lessons of COVID-19 should not be forgotten by scientists and lack of preparedness and slow development of combination drugs has brought us to this doom. A malarial drug with no known retroviral drug properties, namely hydroxychloroquine, and interestingly this molecule docks very well with neuraminidase viral protein. the long chain of the drug molecule seems to be accommodating in the large protein cavity giving chance for surprisingly higher binding constant, which could have become basis had our approach has been followed. If we can find a simple small molecule which interacts in neuraminidase protein binding site along with hydroxychloroquine, we will be having a combination drug to combat the COVID-19.

Assay for confirmation of anti-viral activity for COVID-19 neuraminidase enzyme (*ANGIE LACKENBY, VIRUS REFERENCE DEPARTMENT, USA "INFLUENZA MUNANA NEURAMINIDASE ACTIVITY AND INHIBITION ASSAY (FLUORESCENT IC50 ASSAY)" SOP NO. V-6815/01-10***)**

The method to determine influenza virus neuraminidase (NA) activity and sensitivity to neuraminidase inhibitors (NI) uses an enzyme assay with a fluorescent substrate. NA activity and NI sensitivity can be determined using the fluorogenic substrate, MUNANA (2' 2'-(4-Methylumbelliferyl)-α-D-N-acetylneuraminic acid sodium salt hydrate, Sigma- Aldrich catalogue Number: M8639). This substrate is cleaved by NA to yield free 4-methyumbelliferone (Standard available for standard curve Sigma- Aldrich catalogue Number: M1508) and the quantitative increase in fluorescence gives a measure of NA activity. The concentration of drug needed for inhibition of enzyme activity by 50% (IC50) is determined by assay in the presence of NIs. This approach is absolute and the combination drugs inhibiting the enzyme as determined by in silico study will be verified here. The confirmation of experimental inhibition is good enough for COVID-19 Cell line assay and subsequently recommendation for clinical use. The combination drugs individually are used already in clinical practice and currently their approval for clinical studies takes days to weeks and it is the quickest way to identify a drug for COVID-19 or any future influenza based new viruses.

Research papers in this domain by the Dr Phani Kumar Pullela's group

1. **Narayanan, Manoj M, PK Pullela et al "Design of multiligand inhibitors for the swine flu H1N1 neuraminidase binding site" Advances and applications in bioinformatics and chemistry 6, 47-53 (2013).**
2. **McCullough, C.R., Pullela, P.K., et al "13C-Methyl isocyanide as an NMR probe for cytochrome P450 active sites" J Biomol NMR 43, 171–178 (2009)**
3. **H Yao, CR McCullough, AD Costache, PK Pullela, DS Sem "Structural evidence for a functionally relevant second camphor binding site in P450cam: model for substrate entry into a P450 active site" Proteins: Structure, Function, and Bioinformatics 69 (1), 125-138 (2007).**
4. AD Costache, PK Pullela, P Kasha, H Tomasiewicz, DS Sem "Homology-modeled ligand-binding domains of zebrafish estrogen receptors α, β1, and β2: from in silico to in vivo studies of estrogen interactions in Danio rerio as a model system" Molecular Endocrinology 19 (12), 2979-2990 (2005).
5. PK Pullela, P Rangappa, SR Alapati, PV Subbarao, JA Bibbs "1, 4-dihydropyridine and pyridine compounds as calcium channel blockers" US Patent 6,852,742.
6. PK Pullela, P Rangappa, SR Alapati, PV Subbarao "Substituted dihydropyrimidines, dihydropyrimidones and dihydropyrimidinethiones as calcium channel blockers" US Patent 7,687,511.
7. **R Paramashivappa, PK Pullela, PVS Rao, AS Rao "Design, synthesis and biological evaluation of benzimidazole/benzothiazole and benzoxazole derivatives as cyclooxygenase inhibitors" Bioorganic & medicinal chemistry letters 13 (4), 657-660 (2003)- ONE OF THE HIGHEST CITED PAPER IN "NSAID" SEGMENT.**
8. **Phani Pullela et al "The Synthesis, Characterization, and Application of 13C-Methyl Isocyanide as an NMR Probe of Heme Protein Active Sites" Cytochrome P450 Protocols 51-59 (2013).**
9. M. Chandrappa et al "Fe3O4@SiO2 magnetic nanoparticles for bulk scale synthesis of 4'-chloro-2,2':6',2"-terpyridine" Chemical Papers 71 (12) 2445–2453 (2017).
10. M Chandrappa et al "Efficient bulk scale synthesis of popular pesticide synthon: tetrachlorothiophene" Catalysis, Structure & Reactivity 3, 138-145 (2017).

9. The proposed work plan- give details of project components and work plan of each component with time line

OBJECTIVE WISE ACTIVITIES & TIMELINES			
Objective 1: Combination drug leads for COVID-19			
Activities	***Month Of Start Of Activity***	***Month Of End Of Activity***	***Deliverables***
Chemdraw or ACS Chemsketch used chemical drug structure drawing and processing	1	2	There are about 400 chemicals structures of which about 40-50 are potential antibiotics and antivirals. The molecules need to be drawn or taken for Pubmed and validates, identify enough chemical linkage locations in each molecule
COVID-19 specific protein target identification and processing of the same from PDB, setting, the DockThor for initial docking, standardization of the protocol for docking, orientation of docking box, time for each docking	2	3	The protein target docking with potential drug molecules and providing information electrostatic interaction, docking energy & amino acid specific interaction in solvated and regular conditions
Adoption of 3 mentioned processes in docking, methyl based connected drug combinations, secondary binding site identification and docking and finally docking different combinations of drugs on different virus protein targets with COVID-19 focus	3	4	This should lead to identification of drug combinations which can work for clinicians and narrows down the potentially high thousands of combinations to few with an associated logic
Objective 2: Validation of the efficient approach for COVID-19 drug combination identification			
Activities	***Month***	***Month***	***Deliverables***

	Of Start Of Activity	***Of End Of Activity***	
combinatorial approach of screening the combinations of drugs with no bias of the binding	5	6	In relation to COVID 19, we would have covered most protein targets and potential drug combinations
Scoring of different attributes like docking energy, electrostatic potential, amino acid interactions	6	8	A detailed fool proof system with weighted average of individual indicators/ parameters and their contribution as a generic tool for determining the drug combinations beyond neuraminidase & Hemagglutinin targets
Objective 3: Comprehensive process for combination drug identification for any bacteria, virus, drug resistant strains & targets			
Activities	***Month Of Start Of Activity***	***Month Of End Of Activity***	***Deliverables***
Creation of generic protocol with significant automation	8	10	This is a software program which takes input of the first phase of process developed for newer drug resistant or infectious disease targets and will have functional system to see its operational
streamlining the software and server systems to dock different combination drugs including download of pdb structures preparation of the pubmed structures	10	11	By this time the system just requires will need only the Pubmed link, PDB code and the allied tools in the system will be triggered to obtain docked structures and results
automated ascertaining the weighted	11	12	Once the scores are assigned, the

scores to determine combination drug scores			developed system will help determine combination drugs for any particular disease with minimal time, usually with in a week or ten days of identifying the target protein structure

10. Networking of institutions/ institutions along with their capabilities

Dr Phani Kumar Pullela: Did PhD in Medicinal chemistry in the year 2003, did 4.5 years of postdoc with Dr Daniel Sem at Marquette University in Bi ligand approach for drug development. Dr Pullela worked 7 years in biomedical industry and his groups product is commercially available in market for diagnosis of Swine flu, Tuberculosis, etc. He has two land mark research papers in this domain reputed journals a discovery of second binding site on Cytochrome P450cam by NMR b Identified that the chemicals namely camphor, methyl salicylate and menthol form a tri ligand, which has binding capacity equal to that of Oseltamivir for the Neuraminidase protein of H1N1 virus. ***Dr. Pullela is recipient of DBT's Biotech Product, Process Development & Commercialization award 2017, the highest honor for Biotech product development in India, through hands of Honorable President of India***.

Figure 6: Dr Pullela's DBT Biotech product, process development & commercialization Award 2017

11. Deliverables with Quarterly Milestones

Milestone 1: COVID-19 combination drug identification for clinicians

Milestone 2: Combinatorial screening of different drug molecules for combination drugs and going beyond the typical neuraminidase & hemagglutinin- a generic tool for existing & newer viral infections

Milestone 3: Automated screening of drug combinations to derive possible drug combinations for clinicians for any given disease protein target

Milestone 4: Consolidate & repurpose data & submission of report

12. Milestones to be monitored (give for each 3 months)

SNo	***Milestone Name***	***Month of end of activity***	***Description***
1	COVID-19 combination drug identification for clinicians	3	By end of 3 months we will deliver combination drugs with most potential for clinicians for COVID-19. Without this tool, clinicians currently have to try different drug combinations with no logic and are currently trying directly on humans. This is not sustainable because of the potential 20000 combination drugs with 2 active molecules and ours is probably the only scientific approach to narrow this number
2	Combinatorial screening of different drug molecules for combination drugs and going beyond the typical neuraminidase & hemagglutinin- a generic tool for existing & newer viral	9	With respect to COVID-19, a detailed multiple drug targets both on the same target protein as well as on the virus itself with combination drugs need to be studied. This will be comprehensive will broaden the scope of designing combination drugs for any target with COVID-19 as example.

	infections		
3	Automated screening of drug combinations to derive possible drug combinations for clinicians for any given disease protein target	11	The automated screening process is more to do with software-oriented approach wherein a server is used to handle the process of identifying chemical drugs from the database, protein preparation and finally obtaining, sorting and analyzing the obtained docked results to arrive at the optimum combination drugs. this final step will complete the study and create a useful functional tool which can help newer drug identification, designing as well as understanding the process of combination drugs for both existing diseases as well as newer/ future infections.
4	Consolidate & repurpose data & submission of report	12	We will be filing patents, extract data for commercial exploitation for medicinal chemistry and relate information for combination drugs for COVID-19 as well as other infectious disease protein drug targets.

13. IPR (Briefly project the development of new IPR from this project)

Intellectual property: we will be using any of the existing IPR of any firm. The proposed softwares are freeware permitted for academic use and the drug combinations are for clinicians and public to know. The confidential or commercial data we generate is with the methylene group connected docking data, which allows new drug creation and it is sold to drug companies for us to generate revenue for the research group and eventually for the bioinformatics startup to be generated.

Combination drug data: *For clinicians to use to save lives:* Methylene group tagged multi ligand docking and interaction & allied information; *For us to help* new drug development for companies and it will be sold to R&D.

14. Leadership perspective- Briefly describe the global positioning of the technology and resultant leadership position and economic benefits to the country

Experimental design for drug resistant & other infectious diseases this will be delivered in next six months of the project: This potential and cost-effective approach resulting in combination drug possibilities for clinicians should not be stopped with COVID-19 and eventual adaption to other infectious diseases is needed. By doing this, we not only create a pipeline of combination drugs for clinicians to try for mutated and drug resistant bacteria and virus, but also expand our ability to react for newer kind of pandemics and epidemics. The Swine flu, SARS, MERS, Ebola and COVID-19 has taught us that the evolution span of new endemics is decreasing and soon, every 1-2 years, we should expect newer diseases for which drug combinations are needed to combat and our proposed project can be extended to keep us prepared.

15. Viability analysis

Preliminary work done & confidence of delivering the committed milestones

1. The neuraminidase protein is common for any viral infection and is the gene, which is rarely mutated. even if it is mutated, from the chemical drug perspective it is few amino acids in binding site and does not alter our approach of finding combination drugs.

2. Our results for H1N1 indicates that the over the counter menthol, methyl salicylate and camphor can bind reversibly as a combination drug to slowdown the viral infection. This was found to be true and a simple VICKS inhaler/ vaporub has these compounds and is attributed as one of the reasons for swine flu control in India. This particular multi drug combination is meant for reversible binding and hence will slow down the actual swine flu infection and eventually prevent lung cells being infected. A similar approach for COVID-19 will work too and our proposed project will take this multiligand docking approach to discover new combination drugs.

3. The important aspect to note is that the small molecule combination has the efficiency similar to that of Oseltamivir, zanamivir and sialic acid and none of these three molecules have any affinity whatsoever for neuraminidase protein.