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Drug repurposing with reference to veterinary therapeutics

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Abstract
Drug repurposing and repositioning is a process of identifying new therapeutic use (s) for the available and existing older drugs. Therapeutically, this has become a popular and effective strategy in recent years, in discovering or developing drug molecules with new pharmacological action and therapeutic indications. The drug repurposing strategy is efficient, economical and less time consuming and is thought to be an effective alternative approach to traditional drug discovery process. With the discovery of novel biological targets, the drug repositioning would maximize the success rate of therapy. Drug based vector control is a new strategy that involved administration of an oral insecticidal drug to a human population at risk to kill the insect vector on blood feeding, thereby reducing the vector population and preventing disease transmission. Isoxazolines category of veterinary drugs viz: fluralaner and afoxolaner have been repurposed for control of vector-borne human diseases like malaria and Zika fever. Veterinary antiparasitic drugs of benzimidazole carbamates and halogenated salicylanilides were repurposed and "double repositioned" for human cancers. Using a novel heterogenous label propagation (Heter-LP) algorithm, drugs such as glibenclamide (anti-diabetic), ipratropium, salbutamol (bronchodilators) and carbidopa (anti-parkinson) were successfully predicted as suitable alternatives for treatment of E. coli mastitis in dairy cattle. While the pharmaceutical industry has explored drug repositioning to identify novel treatments for diseases, this work has been hampered by the lack of a fundamental and systematic approach.

Keywords: Drug repurposing, repositioning, veterinary

Introduction
Drug repositioning (DR) is also known as drug repositioning, drug retasking, drug reprofiling, drug rescue, drug recycling, drug redirection, and treatment switching. It identifies new pharmacological indications from old / existing / failed / investigation / already marketed / FDA approved drugs / prodrugs and treats diseases other than the original / intended therapeutic use of the drug. It can be defined as the process of applying a newly developed drug to do so. It is to establish new therapeutic uses for known medicines, including approved, discontinued, experimental medicines [28].

Traditional drug discovery is a time-consuming, labor-intensive, very costly and risky process. New drug repositioning approaches may be used more than traditional drug discovery programs by reducing high economic costs, longer development times, and increased risk of failure. An additional reduction in the risk of failure if a traditional drug discovery program encounters a failure rate of approximately 45% due to safety or toxicity issues, saving up to 5-7 years in average drug discovery time. In recent years, drug repositioning strategies have gained a lot of momentum, with reused drugs accounting for about one-third of new drug approvals and now accounting for about 25% of the pharmaceutical industry's annual sales [18].

Approximately 30% of the drugs and biologics (vaccines) approved by the US Food and Drug Administration (FDA) have been found to be relocated drugs. Recent estimates suggest that the pharmaceutical industry will raise the reused pharmaceutical market to US $ 24.4 billion in 2015 and grow up to US $ 31.3 billion in 2020. The first example of drug relocation was accidental discovery / observation in the 1920s. After about a century of development, other approaches have been developed to speed up the drug switching process. Some of the most successful and well-known medicines born from the DR approach include sildenafil, minoxidil, aspirin, valproic acid and methotrexate [2]. For example, sildenafil, originally developed to treat hypertension and angina, is now used to treat erectile dysfunction.

Traditional drug discovery in veterinary

Pipeline stage for commercialization of veterinary drugs is that the width of the pipeline reflects the number of connections at a particular stage in the pipeline. Successful promotion of a candidate can take 5 to 15 years.

Discovery, Development, and Registration process. Each company has its own internal organizational structure and commercialization approach, but generally has three different research phases. The initial phase is the discovery phase when a new entity is identified. Next is the development stage. At this stage, we collect additional information to decide whether to move the drug to the enrollment stage. At this stage, research is conducted to meet the quality, safety, and efficacy approval criteria set by the regulatory agency [27].

Discovery Phase

The company first conducts a marketing assessment to identify unmet animal health needs that can be addressed and yield a satisfactory return on investment. This assessment guides scientists in looking for new chemistry, new antigens, or other innovative techniques [12]. Chemistry laboratories can synthesize a variety of similar molecules and analogs, and provide fermentation-derived materials that are evaluated by screening against key targets of interest. This process determines structure-activity relationships and finds lead candidates. Preliminary adsorption, distribution, metabolism and excretion (ADME) studies can also be used from time to time. Innovation "checklists" typically include intellectual property (ie, patentability), ease of manufacture, early proof-of-concept studies *in vitro* and *in vivo*, drug active ingredient (API) stability, and animal safety. Includes gender and preliminary considerations and toxicology research. Discovery and preclinical studies are usually not performed according to Good Laboratory Practice (GLP) requirements [7].

Development Phase

A company decides to pursue a new chemistry or technique based on an initial assessment, and additional research is undertaken to further characterize the behavior of the compound. Preliminary assessments are being made on where and how potential products are manufactured. Additional information is also available for market valuations and other business considerations. Internal business decisions may be made to encourage candidates to study the more expensive registration phase. Drug sponsors will contact the Center for Veterinary Medicine (CVM) to open a new veterinary drug review file, which will formally initiate the drug approval process [7].

Registration Phase

This phase begins when studies generally approved by CVM are conducted under the requirements of Good Clinical Practice, GLP, or Good Manufacturing Practice (GMP). All data collected during these studies will be used for information gathering purposes to meet the requirements of the New Veterinary Drug Application (NADA). These studies require GMPAPI materials, except for total residue studies. Needless to say, robust and validated analytical and bioanalytical methods are essential to support these studies. From this point on, most protocols for enrollment stage studies are typically submitted to the CVM for approval. This is not a submission requirement, but it does help avoid regulatory changes, reviewer changes, or simple misunderstanding surprises. CVM approval does not guarantee that the data generated from the matched protocol will support the safety or efficacy of the tested compound. Toxicity studies following the CVM and Veterinary International Harmony Guidelines include target animal safety, genetic toxicity studies, 90-day chronic rodents studies, 90-day chronic non-rodents studies, rat 2nd generation reproductive studies and teratogenicity testing. The results of pilot and preliminary total residue studies are often used to assist in the design of the final toxicological study submitted. Toxicology work can take two to five years to complete. One of the main differences in the development of veterinary and pet medicines is that food safety data for the target species is required for both livestock and pets, but data is required for only one rodent species and is a poison. Scientifically, non-rodine species and target species must meet human food safety requirements when submitting food animals [5].

NADA is a data package sent to CVM for review and approval. The sections of NADA are: Effectiveness, Target Animal Safety; Chemistry, Manufacturing and Management; Environmental Assessment; Human Food Safety (Eating Animals Only); Freedom of Information Summary; Labeling; and all other information. If sponsors wish, they can submit all information about their medication to NADA. However, the FDA / CVM also has provisions for submitting the Veterinary Master File "VMF". This corresponds to the drug master file for human health. VMF is designed to maintain the confidentiality of the manufacturing information of API, which is a compound that provides pharmacological activity. Therefore, you can refer to VMF in the Chemistry, Manufacturing, and Control section of NADA. Alternatively, each section can be submitted on a "step-by-step" or "on-time" basis to facilitate the review and approval process. The last technical section is usually the "All Other Information" section. When all technical sections are completed as part of the step-by-step review process, the company submits the final step. Management NADA contains only a copy of the Completion Notice of the Specialist Department of each Specialist Group, a copy of the finally agreed label, and a complete free copy of the information. At this time, veterinary drug application fees are paid under the Veterinary Drug Usage Fee Act. The FDA then completes the review and makes a decision. Once this is complete, the FDA will grant approval. The final approval will first be published as a Federal Register Notice and then in the official record of the Code of Federal Regulations. At this point, sponsors are typically looking at the types of studies and post-approval data that can be performed on new label claims, such as marketing support data and new prescriptions. These studies are important in the light of the enactment of clarifications for anti-drug drug use and provide clarification to the treating veterinarian if he wishes to consider unlicensed use of approved drugs. However, these studies are outside the scope of the approved label and should not be used for marketing purposes [7].

After approval of food animals, two reports must be submitted to FDA / CVM. The first is a drug experience report that contains information related to drug marketing. This includes annual sales volume, recent advertising and promotional materials, approved labels, and up-to-date information on adverse events from the sponsor's Pharmacovigilance program. New information related to drug use. For the first two years after the first registration, it will be sent to the

CVM every 6 months and then every year thereafter. Another report is the Minor Changes and Stability Report (also known as the Annual Report). As the name implies, this report provides information on minor changes in manufacturing and control information for approved use, as well as the results of ongoing drug and API stability studies. Both reports are submitted annually within 60 days of the date of approval of registration [7].

Traditional drug discovery vs. Drug repurposing

Drug repositioning has several advantages over traditional drug discovery approaches. Significant reductions in R&D time are seen compared to traditional drug discovery programs. With traditional approaches an estimated 10-16 years are spent developing a new drug, whereas with DR the estimated time is 3-12 years. Drug repositioning strategies cost just $1.6 billion to develop new drugs, while traditional strategies cost around $12 billion to develop new drugs. Moreover, it takes only one to two years for researchers to identify new drug targets, and on average about eight years to develop repositioned drugs. A repositioned drug does not require the initial 6–9 years typically required for the development of new drugs by traditional process, but instead enters directly to preclinical testing and clinical trials, thus reducing the overall risk, time and cost of development. Reports suggest that repurposed drugs require approximately 3–12 years for gaining approval from FDA and/or European Medicines Agency (EMA) and at reduced 50–60% cost. At the beginning of a repositioning project, a range of pre-clinical (pharmacological, toxicological, etc.), and clinical efficacy and safety information is already available, as the candidate drug has already undergone through the early stages of drug development such as structural optimization, preclinical and/or clinical trials, in addition to the possibility of the candidate drug being an approved drug, having its clinical efficacy and safety profile. In this way, there is a reduction of the risks associated with failures in the early stages of development, which are high in traditional approaches, as well as a significant reduction of cost with the possible increase in clinical safety and therefore, high success rate. Due to the availability of previously collected pharmacokinetic, toxicological, clinical and safety data at the start of a repurposing development project, the advantages that are encountered with drug repurposing over traditional drug discovery approach are reduced time of development, lower cost of development and reduced risks of failure in the clinical development. It has been estimated that the time required for development of a repositioned drug varies from 3 to 12 years (which is about 10–17 years in traditional discovery program) with substantially lower costs, which ensures the repositioning company's significant savings in terms of time and capital. The average cost required to bring a new drug to market is USD 1.24 billion by traditional drug development process, whereas in drug repurposing it costs around ≤60% expenditure of traditional drug discovery. Some other advantages are as follow. The primary focus of traditional discovery program is to discover drugs to treat chronic and complex diseases, whereas by drug repositioning approach, development of drugs for rapidly emerging and re-emerging infectious diseases, difficult to treat diseases and neglected diseases (NTDs) are focused. Due to the availability of bioinformatics or cheminformatics approaches, huge omics (proteomics, transcriptomics, metabolomics, genomics etc.) data and database resources, disease targeted-based repositioning methods can be used to explore the unknown mechanisms of action (such as unknown targets for drugs, unknown drug–drug similarities, new biomarkers for diseases etc.) of known/existing drugs [18].

Strategies of drug repurposing

DR has two main strategies: on-target and off-target. On-target DR applies known pharmacological mechanisms of drug molecules to new therapeutic indications. In this strategy, the biological target of the drug molecule is the same, but the disease is different. For example, in the relocation of minoxidil, the target profile is observed because the drug acts on the same target and produces two different therapeutic effects. Minoxidil was converted from a vasodilator that lowers blood pressure for hair loss. As an antihypertensive vasodilator, minoxidil has the property of dilating blood vessels, opening potassium channels and allowing more oxygen, blood and nutrients to reach the hair follicles, a pharmacological effect of androgenetic alopecia (male pattern baldness) [18].

On the other hand, in the off-target profile, the pharmacological mechanism is unknown. Drugs and drug candidates act on new targets outside the initial scope of new therapeutic indications. Therefore, both goals and indications are new. Aspirin is a good example of an off-target profile. Aspirin has traditionally been used as an NSAIDs in the treatment of various pain and inflammatory conditions. It also suppresses blood coagulation (coagulation) by inhibiting the normal function of platelets (antiplatelets). Therefore, it is used to treat heart attacks and strokes. Another new use of aspirin in the treatment of prostate cancer has also been reported [18].

Approaches of drug repurposing

There are two alternative and complementary approaches to drug repositioning. One is an experimental approach and the other is an *in silico*-based approach.

The experimental approach is also known as activity-based rearrangement. This refers to screening the original drug for new pharmacological indications based on experimental assays. This includes protein target-based and cell/ biological-based screening in *in vitro* and / or *in vivo* disease models without the need for structural information on the target protein. Several approaches to experimental relocation are the target screening approach, the cell assay approach, the animal model approach and the clinical approach [16].

In silico repurposing uses computational biology and bioinformatics / chemoinformatics tools to perform virtual screening of public databases in vast drug / chemical libraries. In this approach, identification of potential bioactive molecules is achieved based on molecular interactions between drug molecules and target proteins [24].

Over the past few decades, the *in silico* approach has gained widespread popularity and has been a remarkable success in drug discovery programs. Many pharmaceutical companies and drug discovery laboratories have a large amount of information on the chemical structure, protein structure, and pharmacophore model of bioactive compounds, so they can be used as incilico tools for drug discovery from structurally different chemical areas. We have already successfully integrated the technology. Available in the public domain. In addition, *in silico* relocation has several advantages over the experimental approach, such as reduced development time and cost, and reduced risk of failure. The limitation of this

method is that accurate structural information about the drug target is required, and if a protein target is not available, a disease-specific phenotype or genotype profile of the drug is required [17].

In recent years, discovery scientists and researchers have combined *in silico* and experimental approaches to identify new therapeutic indications for existing drugs, called the mixed approach. In the mixed approach, the results of the calculation method are validated by preclinical biological experiments (*in vitro* and *in vivo* tests) and clinical studies. Simultaneous application of computational and experimental methods in a systematic way provides a robust and logical approach to discovering new indications, demonstrating greater efficiency than accidental discovery. In addition, the mixed approach provides an opportunity to develop relocated drugs more effectively and quickly. This approach is reliable but reliable [25].

Methodologies of drug repurposing

The methods used in DR can be divided into three major groups based on the quantity and quality of available pharmacological, toxicological, and biological activity information. These are mainly

A. Drug-oriented
B. Target-oriented
C. Disease/therapy-oriented.

A. Drug-oriented methodology

The structural properties, biological activity, side effects and toxicity of the drug molecule are evaluated. This strategy helps identify molecules with biological effects based on cell / animal assays. This type of rearrangement methodology is based on traditional principles of pharmacology and drug discovery, usually to determine the biological efficacy of a drug molecule without the true knowledge of the biological target. Research will be conducted. Significant success in DR was achieved with this orientation profile by chance or by clinical observations such as discovery with sildenafil [10].

B. Target-based methodology

This includes *in vivo* screening or high-throughput virtual screening (vHTS) of drugs or compounds from drug libraries / compound databases such as ligand-based screening and molecular docking, followed by high-throughput and / or high-content *in vitro* and *in vivo* included. Screening of drugs for selective protein molecules or biomarkers of interest (HTS / HCS). Because most biological targets directly represent the pathway / mechanism of the disease, the success rate of drug discovery with this method is significantly higher than with drug-oriented methods [15].

C. Disease/therapy-oriented methodology

In DR, it is relevant when more information about the disease model is available. In this case, DR is from proteomics (disease-specific target protein), genomics (disease-specific genetic data), metabolomics (disease-specific metabolic pathway / profile), and phenotypic data (disease-specific metabolic pathway / profile). It may be a disease and / or treatment that is guided based on the availability of information. Off-target mechanisms for disease processes, pharmacological targets, disease pathways, pathological conditions, side effects and side effects, etc.). Therefore, it is necessary to establish specific disease networks, detect gene expression, examine important targets, and identify protein molecules responsible for diseases related to cells and metabolic pathways of interest in disease models [16].

Drug-based phenotypic screening and target-based methods account for more than 50% of FDA-approved small molecule drugs and biologics. Phenotypic drug screening methods identify drug candidates from small molecule libraries through random observations. The target-based method discovers drugs based on known target molecules. The treatment / treatment-based relocation methodology is similar to the disease-based methodology [16].

Resource for drug repositioning

Information	Database
Chemical structure and drug's activities	PubChem Che EMBL Drug Bank Drug Central cMap STITCH
Transcriptional response induced by drugs	Connectivity Map Library of Integrated Network-based Cellular Signatures (LINCS)
Protein structure	Protein Data Bank (PDB) Protein Binding Sites (Pro Bis) Protein-Ligand Interaction Profiler (PLIP)
Protein structure and transcriptional profile and drug's activities	Drug Repurposing Hub Drug Target Commons Open Targets
Clinical information	Repo DB repurpose DB

Repurposed drugs

Drug repositioning is an alternative to traditional drug discovery. Faced with rising market demand, many pharmaceutical companies are developing new or new therapeutic applications from existing / old / available drugs through a faster, lower cost, relocation approach [3].

In a drug discovery program, relocations are typically performed in two main phases, as described below. In the first stage, approved drugs are screened *in silico* for specific disease targets, and in the second stage, the selected identified molecules are further *in vitro* and *in vivo* in the specific disease model of interest. It will be investigated experimentally. Following successful preclinical trials in the second phase of relocation, identified drug candidates are

participating in human clinical trials [18]. Colchicine, a well-known anti-inflammatory drug used to treat gout and pericarditis, is currently undergoing clinical studies for the treatment of patients with COVID-19. This drug has been shown to be effective in preventing pneumonia caused by SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus-2) caused by a large cytokine storm. The antiviral effect of the old antimalarial drug chloroquine (used as a phosphate) against SARS-CoV-2 infection is also being studied worldwide. Studies suggest that chloroquine may be beneficial in preventing COVID-19 pneumonia caused by coronavirus. According to a recent report from the NIH (National Institutes of Health), clinical trials of the hydroxychloroquine / azithromycin combination for the treatment of COVID-19 patients have already begun. In this combination, both drugs are FDA approved, hydroxychloroquine is an antimalarial drug, and azithromycin is an antibacterial antibiotic. Favipiravir, an antiviral drug intended to treat influenza, is currently undergoing Phase 2 / Phase 3 clinical trials in COVID-19 patients worldwide (China, Japan, US, India). Glenmark has begun a phase 3 trial of favipiravir in the treatment of COVID-19 patients in India. An anti-retroviral drug under investigation called remdesivir (originally developed by Gilead Sciences to treat Ebola hemorrhagic fever but failed in clinical trials) is also available in China, the United States, for the treatment of COVID-19 patients. It is in clinical trials in several countries, including the United Kingdom and India. Clinical trials of favipiravir, remdesivir, and colchicine are currently being conducted in India by the CSIR [26].

A fixed-dose combination drug called lopinavir / ritonavir, previously approved for the treatment of HIV / AIDS under the brand name Kaletra, is currently being studied in several countries for the treatment of COVID-19 patients. This active ingredient combination was studied in Thailand with the influenza drug oseltamivir (Tamiflu) to treat infections caused by SARS-CoV-2. A clinical trial of an anthelmintic drug called ivermectin (traditionally used as an approved treatment for worm infection) for the treatment of COVID-19 has been shown to be effective *in vitro* for SARS-CoV-2 infection in Monash. A university in Melbourne, Australia that has been held in multiple parts of the world after sexual success. A clinical trial of tocilizumab, an IL-6 receptor antagonist (sold under the brand name Actemra) used to treat inflammatory diseases such as rheumatoid arthritis, is also being conducted to treat patients with COVID-19 [20].

Table1: Veterinary repurposed drugs

Drug	Pharmacological category	Original indication	New indication	Status of development	Possible mechanism
Amphotericin B (AMB)	Anti-fungal antibiotic	Fungal infections	Leishmaniasis	Already developed	Disruption of membrane sterols impairing barrier permeability
Aspirin	NSAID	Pain and inflammation	Cardiovascular diseases (anti-platelet) Prostate cancer	Already developed Under development	COX-1 inhibition (platelet) Nuclear factor kappa b inhibition (NFκB) (prostrate cancer)
Amantadine	Anti-viral	Influenza	Parkinson's disease	Under development	NMDA antagonism – increase dopamine release and reduce reuptake
Bromocriptine	Dopamine receptor antagonist	Parkinson's disease	Diabetes mellitus (type 2)	Under development	Increased plasma glucose, free fatty acids and triglycerides through elevated hypothalamic drive
Celecoxib	COX-2 inhibitor, nsaid	Inflammation	Breast and colon cancer	Under development	Inhibition of estrogenic receptor (breast cancer), induction of PP2A
Chloroquine	Anti-malarial	Malaria	COVID-19	Under development	Inhibiting quinine reductase-2, sialic acid biosynthesis
Cimetidine	H2 receptor Antagonist	Gastric ulcer	Breast, lung and prostate cancer	Under development	Decrease in E- selectin on endothelial cell and NFκB
Crizotinib	ALK inhibitor	Lymphoma (under Clinical trial for ALCL)	Non-small cell lung carcinoma	Already developed	Tyrosine kinase inhibition
Colchicine	Anti-inflammatory Agent	Gout (gouty arthritis)	Pericarditis COVID-19	Already developed Under development	Inhibit tubulin polymerization
Digoxin	Cardiotonic	CVDs such as heart Failure	Prostate cancer	Under development	Increase in intracellular Ca2+ and apoptosis
Dimethyl fumarate	Anti-allergic	Psoriasis	Multiple sclerosis (MS)	Already developed	Nrf2 dependent anti-inflammatory pathway
Disulfiram	Acetaldehyde Dehydrogenase inhibitor	Chronic alcoholism	Cancer	Under development	Inhibit proteasome activity and inhibit cellular signaling pathway
Duloxetine	SSNRI	Depression	Generalized Anxiety disorder, Fibromyalgia, chronic Musculoskeletal pain, Neuropathic pain	Already developed	Reuptake inhibition of serotonin and norepinephrine
Everolimus	Immune Suppressant	Immune suppressant	Pancreatic Neuroendocrine Tumors	Already developed	Inhibits mTOR (kinase)
Favipiravir	Anti-viral	Influenza	COVID-19	Under	Inhibit RdRp complex of SARS-

				development	COV-2
Fluoxetine	Anti-depressant	Depression	Premenstrual Dysphoria	Already developed	SSRI
Gabapentin	Anti-epileptic	Epilepsy	Neuropathic pain	Already developed	Inhibiting N-type calcium channels of presynaptic terminals of dorsal root ganglion
Galantamine	AChE Inhibitor	Neuromuscular Paralysis	Alzheimer's disease	Already developed	AChE inhibition
Hydroxychloroquine	Anti-malarial	Malaria, Rheumatoid arthritis	COVID-19	Under development	Blockade of sialic acid receptor, clevage of ACE- II, cytokine storm
Imatinib	TKI (anti-cancer)	Anti cancerous Chronic myeloid leukemia Acute lymphocytic leukemia	Gastrointestinal stromal tumor	Already developed	Tyrosine kinase inhibitor
Isoniazid	Anti-tubercular	Tuberculosis	Certain types of Tumor	Already developed	Increase in ROS generation Induce cancer cell apoptosis
Itraconazole	Anti-fungal	Fungal infections	Cancer like non-small cell lung carcinoma (anti-angiogenic)	Under development	Inhibition of hypoxia- inducible factor 1
Ivermectin	Anthelmintic (anti-parasitic)	Scabies, river Blindness, Helminthiasis	COVID-19	Under development	Decrease IL-6, TNFα, binding to RdRp
Metformin	Anti-diabetic	Diabetes mellitus (type 2)	Breast and colon Cancer, cardiovascular diseases Polycystic ovary syndrome	Under development	Decrease in insulin growth factors, ROS (cancer) Increased eNOS (CVS) Reduced lipid, androgens (PCOS)
Methotrexate	Antimetabolite (anti-cancer)	Cancer	Psoriasis, Rheumatoid arthritis	Already developed	Increase in adenosine levels (RA) Antiproliferative and immunomodulatory action (psoriasis)
Mifepristone	Antiprogestin	Termination of Pregnancy in Combination with Misoprostol	Cushing's syndrome	Already developed	Blocking glucocorticoid receptor
Minoxidil	Vasodilator (anti-hypertensive)	Hypertension	Androgenic alopecia	Already developed	Vasodilation – improved circulation to hair follicles
Orlistat	Anti-obesity agent	Obesity	Cancer	Already developed	Inhibit FAS (fatty acid synthase)
Propranolol	B-blocker	Hypertension	Migraine	Already developed	Inhibiting arterial dilatation, central mechanisms in brain
Remdesivir,	Anti-viral	Influenza, Ebola (failed in clinical trial)	COVID-19	Under development	Inhibiting RdRp of SARS-COV-2
Retinoic acid	Retinoids (synthetic vitamin a)	Acne	Acute leukemia	Already developed	Increase retinoic acid receptor β 2
Ribavirin	Anti-viral	Viral infection such As respiratory syncytial virus, Hepatitis C Infections	Cancers like Leukemias and Lymphomas	Under development	Stimulating dopamine receptor
Ropinirole	Anti-Parkinsonian drug	Parkinson Disease	Restless leg Syndrome	Already developed	Stimulating dopamine receptor
Sildenafil	PDE inhibitor	Angina pectoris, Pulmonary arterial Hypertension	Erectile dysfunction already	Already developed	Vasodilation, increase in cyclic -GMP
Simvastatin	Hypolipidemic	CVDS	Lung cancer	Already developed	Inhibiting NFκB, Decrease in cyclin D1 and CDKS, mmp-9
Sunitinib	TKI (anti-cancer)	Imatinib-resistant GIST Renal cell carcinoma	Pancreatic Neuroendocrine Tumors	Already developed	Inhibiting VEGFR and PDGFR decrease angiogenesis
Tamoxifen	Anti-estrogen (anti-cancer)	Breast cancer, Anticancer	Systemic lupus Erythematosus Neglected tropical diseases like Leishmaniasis (in Combination with Miltefosine)	Already developed Under development	Interfering with IPC- sphingolipid metabolism
Thalidomide	Immune Modulator	Immunomodulation, Morning sickness	Multiple myeloma, Leprosy	Already developed	Anti-inflammatory through selective inhibition of the pro-

		(withdrawn)			inflammatory cytokine TNF-alpha produced by monocytes
Tocilizumab	Il-6 inhibitor (immune modulator)	Rheumatoid arthritis	COVID-19	Under development	Inhibiting IL-6 receptor, cytokine storm
Topiramate	Anticonvulsant	Convulsions	Inflammatory bowel disease	Already developed	Upregulation of transient receptor potential vanilloid 1 (TRPV1) receptors and ion channels Acts on NFκB signaling, inflammatory response
Valproic acid	Anti-epileptic	Epilepsy	Manic depression (bipolar disorder), Migraine headache	Already developed	Increase GABA
Valsartan	ARB (anti-hypertensive)	Hypertension, heart Attack	Alzheimer's disease	Already developed	Preventing Amyloid B deposition in the brain and attenuate cognitive impairment

Veterinary repurposed drugs

1. Isoxazoline veterinary drugs for the control of vector-borne human diseases

Isoxazoline is an oral pesticide currently approved for the control of ectoparasites in livestock. Fluralaner and afoxolaner rapidly killed Anopheles mosquitoes, Aedes, Culex pipiens and sandflies after feeding drug-added blood meals with IC50 values in the range of 33-575 nM into strains with existing resistance to common insecticides. On the other hand, it was completely effective. Based on the axonometric scaling of preclinical pharmacokinetic data, they were single humans at 260 mg (IQR, 177-407 mg) for afoxolaner and 410 mg (IQR, 278-648 mg) for fluralaner. Predicted that the average dose may have an insecticidal effect 50-90 days against mosquitoes and sandflies. Computer models have shown that seasonal high doses of such single-dose drugs to some of the local population dramatically reduce clinical cases of Zika fever and malaria in endemic situations. Therefore, isoxazoline represents a promising new component of drug vector regulation [13].

2. Veterinary Antiparasitic to Human Anticancer

Veterinary antiparasitics such as benzimidazole carbamates (BZ) and halogenated salicylanilides (HS) as novel anticancer drugs. These agents have revealed pronounced anti-tumor activities and gained special attention for "double repositioning", as they are repurposed for different species and diseases simultaneously, acting via different mechanisms depending on their target. BZ carbamates (albendazole, fenbendazole, flubendazole, mebendazole, triclabendazole, parbendazole, oxibendazole, and ricobendazole) and HS drugs (closantel, niclosamide), when administered alone or in combination, elicit anti-tumor activities demonstrated by various biological actions, such as inducing apoptosis and autophagy; reducing cell viability, migration and invasion; disrupting tubulin polymerization; inducing differentiation and senescence; reducing angiogenesis; impairing glucose utilization; arresting the cell cycle; and targeting several key oncogenic signal transduction pathways. These agents induce minimal cytotoxicity in normal cells, but comparatively high cytotoxicity in tumor cells, exerting cancer cell-specific selectivity.

Their significant effect on cancer cell lines, BZ carbamates and HS drugs have shown antitumor effects in *in vivo* animal cancer cell models, including reduced tumor growth and vessel formation, reduced metastasis, prolonged overall survival, and progression-free survival. However, clinical trials remain a major bottleneck for successful veterinary anthelmintic relocation in oncology due to inadequate and useful data. Another obstacle to the successful rearrangement of veterinary antiparasitic drugs as anticancer agents is related to their physicochemical properties, routes of administration, and inadequate bioavailability. Although the safety of BZ carbamate and HS drugs has been evaluated for veterinary care, their use as anti-cancer agents for human use has not been fully established. Potential activities such as toxicity, side effects and efficacy of recycled drugs against cancer and non-cancer cell populations after short-term and long-term administration guarantee future detailed experimental investigations. Despite limited data, some of these drugs have already been tested in Phase II / III clinical trials for the treatment of cancer. Some patients with late-stage metastatic cancer responded well to albendazole, mebendazole, and niclosamide, showing reduced tumor markers and

metastases, and stabilized disease progression. Ongoing clinical trials will soon become clearer about the potential of BZ carbamate and HS drugs for use in the treatment of primary and metastatic cancers [23].

3. Veterinary antiprotozoal drug for the treatment of *Clostridioides difficile* infection

Clostridium difficile infection (CDI) is the leading cause of nosocomial infections and deaths worldwide. Unfortunately, the need for new, more effective anti-rostridium agents has not been met. Against this background, drug repositioning can be used as a fast and inexpensive method of drug development. As a potential treatment for *Clostridioides difficile* infection, we will evaluate the activity of the antiprotozoal ronidazole. Ronidazole inhibits the growth of clinical *C. difficile* isolates (including NAP1 and toxin-producing strains) at very low concentrations (0.125 µg / mL) compared to metronidazole, a known anti-*Clostridium* compound in the same chemical category showed excellent killing rate. In addition, ronidazole did not inhibit the growth of some symbiotic organisms that occur naturally in the human intestine and play a protective role in the prevention of *Clostridium difficile* infection. In addition, ronidazole was found to be non-toxic to human enterocytes and penetrate a monolayer of colonic epithelial cells (Caco-2) at a slower rate than metronidazole. Finally, when both were tested at a daily dose of 1 mg / kg in a mouse model of *Clostridium difficile* infection, ronidazole outperformed metronidazole. Ronidazole is a potent inhibitor of *C. difficile* growth *in vitro*, a low-concentration fungicide, penetrates enterocytes at a slower rate than metronidazole *in vitro*, and is more potent than metronidazole *in vivo*. Effective against metronidazole-resistant parasite strains. Further investigation is needed as a

potential treatment for *C. difficile* infection [1].

4. Drug Repurposing of Mastitis in Dairy Cattle

Elucidating the biosignature of dairy cow mastitis may open new avenues for drug reuse. A new semi-supervised heterogeneous label propagation algorithm called Heter-LP that applies both local and global network capabilities for data integration is used to potentially identify new therapeutic routes for the treatment of *E. coli* mastitis. Online datastores related to known diseases, drugs, and gene targets, along with other specialized biological information about *E. coli* mastitis, including robust biosignatures, drugs, and important genes with related diseases. Researchers have identified new drugs such as glibenclamide, ipratropium, salbutamol, and carbidopa as potential treatments for *E. coli* mastitis. Predicted associations can be used by pharmaceutical scientists or veterinarians to find commercially effective drugs or combinations of two or more drugs to treat this infection [21].

5. Screening of drug repurposing libraries with nematode motility assays identifies promising anthelmintic hits against *Cooperia oncophora* and other ruminant parasites

Cooperia oncophora accounts for the majority of reports on the development of drug resistance in parasites worldwide. Therefore, a convenient, automated and inexpensive whole biomotility assay for the excreted L3 stage (xL3s) of the ruminant parasite *Cooperia oncophora* has been established using the W Micro Tracker instrument for the reuse of 2745 molecules. The library has been screened. 14 known anthelmintics in this library were picked up on this blind screen and there were 4 new hits. Thonzonium bromide, NH125, physostigmine sulphate, and EVP4593. The four hits were also effective against xL3 from *Ostertagia osteoma*, *Haemonchus contortus*, and *Haemonchus contortus* using the same assay. Cytotoxicity studies have shown that Thonzonium bromide and NH125 (1-benzyl-3cetyl-2-methylimidazolium iodide) have significant cytotoxicity. EVP4593 (N (4)-(2- (4-phenoxyphenyl) ethyl) 4,6-quinazoline diamine) showed strong, widespread anthelmintic activity and high selectivity index. In addition, given its novel and unexplored chemical scaffolding for anthelmintic activity, EVP4593 was an interesting anthelmintic hit for further optimization [11].

6. Canine Lymphoma (LSA): Drug repurposing to aid treatment of canine lymphoma

Lymphoma is one of the most common types of cancer in dogs and there are few treatment options available. Traditional chemotherapeutic agents have been used to extend quality of life, achieve clinical remissions, and slow the progression of cancer, but they can be costly. Dog patients need additional safe and inexpensive treatments. This study reuses antibiotics with data on the usefulness and safety of dogs in the treatment of certain infectious diseases. In addition, studies have shown that the investigational drug reduces the ability of lymphoma cells to reproduce in the laboratory. In the current study, dogs are prospectively enrolled to receive either prednisone alone or in combination with the study drug. This study is available through the Cornell University School of Veterinary Oncology Services and is sponsored by the Cornell University Animal Health Grant Program. Objective: The purpose of this study is to determine whether reused antibiotics can improve the outcome of dogs with large cell lymphoma in combination with prednisone compared to dogs receiving prednisone alone. In addition, you will receive data to identify prognostic factors in dogs with lymphoma treated with cheap oral medications.
(https://www.vet.cornell.edu/hospitals/clinical-trials/drug-repurposing-aid-treatment-canine-lymphoma)

Opportunities and challenges

Unlike traditional drug discovery programs, a complex and time-consuming process with high development costs and risk of failure, drug repositioning reduces the time and cost of drug development. Drug repositioning is also a low-risk strategy. The computational or machine learning approach has significantly improved the performance of drug repositioning. Experimental approaches that provide a direct evidence-based understanding of drug-disease associations compared to computational approaches (eg: target protein-based screening, cell-based assays, animal model trials, and clinical trials). However, in recent years, computational approaches have usually been combined with experimental approaches to identify new indications for older medicines. This is the so-called mixed approach. In this approach, the computational method is validated by biological experiments and clinical trials [18]. The mixed approach to reposition provides a rational and comprehensive study of all possible relocation opportunities, taking into account increased access to available databases and technological advances. In addition, the R & D investment required for drug repositioning is lower than traditional drug research. Therefore, drug repositioning offers many pharmaceutical companies the opportunity to develop drugs with little investment [28]. The mixed approach of DR provides an opportunity for more effective and rapid development of relocated drugs. From a market perspective, many illnesses require treatment of new drugs with potential market demand and economic impact. For example, drug discovery for orphans / neglected diseases offers great potential markets for exploration. Therefore, there is an opportunity to reuse medicines for the treatment of rare, neglected, rare or difficult-to-treat illnesses. There are over 6000 rare diseases that are not properly treated. About 5% of these will be investigated. Rare diseases have great potential markets to explore. Given the high failure rate, high cost, and slow pace of drug discovery and development, diversion of legacy drugs to treat both common and rare diseases involves the use of fewer drug molecules. As a result, it is becoming an increasingly attractive area of research, risk of failure, faster time, and lower development costs [14, 24].

With the advent of technologies such as genomics, proteomics, transcriptomics, metabolomics, and the availability of vast database resources including drug omics data, disease omics data, etc., there are many opportunities to discover drugs through population drug relocation and an integrated effort of all the above methods / approaches. Researchers are currently up-to-date and reliable tools and data for exploring new unknown mechanisms of action / pathways based on disease-specific target proteins / genes and / or specific biomarkers associated with disease progression [28].

Various databases and software are available for genomics, proteomics, metabolomics, and pathway analysis. Several computational strategies have already been developed to improve the speed and simplicity of the conversion process. However, when it comes to drug repositioning, many

challenges come with opportunities. Identifying new therapeutic indications for existing drugs is a major challenge in relocation, but drug relocation is complex, involving multiple factors such as technology, commercial models, patents, investment and market needs. Several challenges, including clinical trial-related issues, including selecting the appropriate treatment area for the drug under investigation. If data from the original drug or drug clinical or preclinical study is outdated or inadequate, the need to conduct a new study from scratch [24].

Barrier to drug repositioning

Despite the benefits of drug repositioning, identifying potential uses for existing medicines still requires significant risky investments. Relocated drugs may not be sufficiently effective in clinical trials, even if safety protocols are adequately adhered to. Here are some reasons:

A. Dose-Dependency

The correct dose of the drug depends on the illness. The prescribed dose of the potential drug is important for its optimal therapeutic effect. Once the relocation is approved, the indications for that appropriate dose should also be investigated through clinical trials [9].

B. Data Availability and Heterogeneity

An open source model has been proposed in response to the growing number of published expression data. Public access to certain types of data. However, clinical patient data is limited and requires extensive editing for direct use and understanding. Due to the non-uniformity of the data, the combination of different data types such as transcriptome data, chemical structure data, clinical literature data, etc. poses another computational challenge for effective drug repositioning [9].

C. Patenting of Drug

If new indications are identified within the same drug category, it is very difficult to patent potential drugs. A systematic collection of patents for drug repositioning, along with a support system to support the extraction of related patents, helps determine patent ownership of potential drug-disease [9].

D. Validation of Drug

Achieving the ultimate success in drug repositioning requires a combination of *in silico* prediction and *in vitro* validation. Various approaches to drug repositioning identify new drug-disease relationships and can be combined with clinical records such as EHR, health insurance records, and physical examination data to effectively identify potential drugs. High-throughput screening of chemicals using *in vitro* or *in vivo* systems may help validate predicted drug candidates. However, most *in vitro* systems differ from physiological conditions, so an *in vitro* cell culture model that resembles the pathology of *in vivo* tissues and diseases should be examined for validation [9].

E. Regulatory and intellectual property issues

Traditional drug development strategies are costly, error-prone, and costly efforts. Therefore, recent drug repositioning has received considerable attention in the discovery of new therapeutic applications with the aim of bringing the drug to market relatively quickly for clinical use. Some regulatory issues commonly encountered in drug repositioning are described below [8]. According to regulatory guidelines, new preclinical and / or clinical trials should be conducted if the data available are inadequate and do not meet the requirements of regulatory bodies such as the FDA and EMA. Another important issue concerns patent registration and intellectual property rights (IPR). There are no provisions regarding the protection of intellectual property in drug research by relocation under the Intellectual Property Law and Patent Law. IP protection for newly placed medicines is limited. IP protection is limited when relocating the drug. For example, the association of several new targeted diseases discovered by repositioning researchers has been confirmed in publications or online databases. However, by law, it is difficult to obtain IP protection for such organizations. IP issues prevent some relocated medicines from being marketed [6]. In addition, some relocation projects need to be canceled, which is a waste of time, money, and a lot of effort. Although many omics and medical databases have been established, large amounts of data may not be valid unless they are from a trusted source, so due to regulatory issues, choose the appropriate approach for relocation, it is still a challenge. Therefore, researchers or manufacturers must strictly adhere to regulatory criteria guidelines for drug discovery with a relocation approach [22].

Conclusion

Traditionally, drug reuse has had a long, documented history of discovering drug molecules, especially through accidental observations. In recent years, it has paved the way for the development of new therapies based on existing / licensed medicines. The more systematic and rational strategic rearrangement of drugs has revolutionized the discovery of drug molecules with unknown therapeutic indications. The approach to drug repositioning is driving market demand as it significantly reduces R & D costs, increases the chances of success, reduces research time, and reduces investment risk. These benefits are beneficial to researchers, drug researchers, consumers and pharmaceutical companies, enabling the application of new approaches to relocation strategies in drug discovery programs for almost all human and veterinary diseases.

Moreover, the use of *in silico* techniques along with the application of structure-based drug design (SBDD) and pharmacophore modeling strategies and artificial intelligence (AI) technology can further accelerate the process of drug purposing in the drug discovery program. In the era of precision medicine, the drug repositioning strategy has become very much useful to establish the unknown mechanism of action of drugs through exploration of novel disease/metabolic/signaling pathways, or off-targets and target-specific mechanisms/ genetic expression profile for even genetic disorders.

Advancement in genomics have provided us with genomic and transcriptomic data in huge quantities using technologies like next generation sequencing, microarray data and transcriptomics, etc. Network biology and systems biology approaches may add additional benefits to unveil such novel mechanisms of actions with through insights into drug-target interaction profile at molecular/genetic level.

For better drug repositioning, more in-depth understanding are required to be executed with integrated approaches between computational and experimental methods to ensure high success rates of repositioned drugs. However, drug

repositioning can be successfully applied to the discovery and development of new drugs with new and powerful therapeutic indications for veterinary and human diseases.

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