In silico Repositioning of Alendronate and Cytarabine Drugs to Cure Mutations of FPPS, HAP, PTPRS, PTPRE, PTN4, GGPPS Gene and Mutant DNA, DPOLB, TOP2a, DPOLA, DNMT, RNA, TYSY, RIR Genes

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Abstract: Osteoporosis occurs because of the Calcitonin-Related Polypeptide Alpha (CALCA) gene. At present, Caltrate600, Boniva and Alendronate are viewed as dynamic drugs to cure osteoporosis. Chronic lymphocytic leukemia occurs because of the ABL1 proto oncogene. Currently, Rituximab, Fludarabine and Cytarabine are utilized as monoclonal antibodies against this ailment. Drug repositioning is a new rising field of reusing previous drugs, safeguarding retired drugs and developing licenses to make lives easy. The main objective of this research was repositioning of Alendronate and Cytarabine in order to use them in other diseases, too. Interactions of each of these drugs with many off-target proteins were identified. Alendronate presented strong interactions with FPPS, Hydroxylapatite, PTPRS, PTPRE, PTN4 and GGPPS. Cytarabine demonstrated strong interactions with DNA and DPOLB. After screening a great number of drugs which are accustomed to cure mutations of those off-target genes and proteins, their ill effects were compared, and it is suggested that Alendronate and Cytarabine have less side effects than different other drugs utilized for the same interacting targets. Both Alendronate and Cytarabine can be repositioned to cure well known carcinomas and different diseases.

Keywords: Alendronate, Chronic lymphocytic leukemia, Cytarabine, Interactions, Osteoporosis, Repositioning.

Introduction
Osteoporosis, one of the most well-known sicknesses, is a systemic skeletal infection, represented by low bone mass and decay of bone tissue, with an ensuing increment in bone delicacy and weakness to crack. The Canadian Multicenter Osteoporosis Study (CaMos) has evaluated the prevalence of osteoporosis in those over the age of 50 to be 21.3% in women and 5.5% in men. Women suffer from osteoporosis more frequently than men. Sex hormones, low estrogen levels, missing menstrual periods or menopause can results in the development of osteoporosis in women. Low testosterone levels can cause osteoporosis in men.
The Calcitonin-Related Polypeptide Alpha (CALCA) gene is mostly involved in the advancement of osteoporosis [23, 29].

At present, Caltrate 600, Boniva and Alendronate are viewed as dynamic drugs to cure osteoporosis. Chronic lymphocytic leukemia (CLL) is the most well-known leukemia on the earth, with a rate of 4.2 out of 100 000 per year. Nearly 70% of the findings are observed in patients over the age of 65. There are no proper treatments for CLL. In CLL, about 30-100% of the bone marrow are destroyed. ABL proto-oncogene is involved in the development of CLL. Currently, Rituximab, Fludarabine and Cytarabine are utilized as monoclonal antibodies against this ailment [8, 23].

FPPS is involved in colorectal cancer. A higher level of FPPS actions and increased mRNA expression are major causes of illness [22]. Hydroxylapatite is the vital part of the inorganic structure in human bone. It has been found to have an inhibitory capability on the development of numerous classes of tumor cells. Changes in Hydroxylapatite result in breast cancer growth [17]. Polymorphism in the human PTPRS and PTPRE gene leads to ulcerative colitis [18]. Pleiotrophin (PTN), or heparin-binding growth related molecule PTN, is overexpressed in some human tumors, e.g., meningioma, glioma, some breast cancers, pancreatic diseases, and in rheumatoid arthritis [25]. GGPPS1 is increased in the cytoplasm of liver tumor cells. HCC patients with cirrhosis had relative higher expression of GGPPS1 [11].

Gastric cancer is a noteworthy reason for worldwide tumor mortality. Hereditary variations in DNA repair because of transformations in the DNA polymerase beta (DPOLB) gene can regulate DNA repair capacity and therefore, have been associated with the danger of inciting gastric cancer [27]. Long non-coding RNAs (lncRNAs) are assumed to play a critical role in tumor genesis and the resulting prognosis and metastasis of hepatocellular carcinoma [14]. Autosomal predominant cerebellar ataxia, deafness and narcolepsy (ADCADN) is a polymorphic disorder, which occurs due to the transformations of the DNMT1 gene [13]. Oxidative DNA damage is incited by oxygen producing elements that results in the development of bladder cancer [30].

DNA Topoisomerase alpha (TOPα) is responsible for DNA replication; over-expression of this gene results in breast cancer [7]. DNA polymerase alpha (POLα) belongs to five different categories and plays its role in DNA replication and repair. The substantial mutations of DNA polymerase alpha result in glandular carcinoma of the colon, thus causing paralysis [16]. Ribonucleotide reductase large subunits (RIR) are required for DNA polymerization and repair; over-expression of RRM leads to the development of non-small cell lung cancer and pancreatic cancer [6]. The TYMS gene is responsible for the production of TYSY protein that regulates folacin metabolism. Transformations in the TYSY protein result in head and neck cancer [20].

Drug molecules do not solely influence their proposed protein targets but also different targets. Drug-protein interactions prompt the disclosure of novel useful targets and pathways. Drug repositioning is a new rising area of reusing previous drugs, safeguarding retired drugs and developing licenses to make lives easy. Docking one drug to a multi-proteins set has been used as a wise methodology. Drug target association is the premise of drug disclosure and configuration but is a time consuming and expensive procedure. The only solution to this issue is to utilize computational ways in order to predict the drug-target interactions and to perform the repositioning of drugs [4, 12, 31].
Materials and methods

Selection of the drugs for repositioning
The work plan was conducted according to a previously published article [19], with some modifications. After screening a great number of medicines used in osteoporosis and chronic lymphocytic leukemia through the Drug Bank database, available at www.drugbank.ca/, Alendronate, which is used to cure osteoporosis, and Cytarabine, used to cure chronic lymphocytic leukemia, were selected.

Drug target interactions predictions
Their interactions with other off-target proteins were predicted by using the Balestra web server, available at http://balestra.csb.pitt.edu/. The drugs were repositioned to be used in several cancers as well as some other diseases. The ADMET properties and toxicity values of Alendronate and Cytarabine were calculated with the assistance of the ADMET Psychem, available at the following free online server www.acdlabs.com/products/percepta/physchem_adme_tox/, and the Protox Drug toxicity server, from http://tox.charite.de/tox.

Docking studies of drugs with different genes
Three-dimensional structures of the proteins of all the mentioned genes and enzymes were downloaded from RCSB PDB, and drug compounds were collected from the Zinc Database, available at http://zinc.docking.org/. Alendronate was docked with FPPS, Hydroxylapatite, PTPRS, PTPRE, PTN4, and GGPPS gene and Cytarabine with DNA, DPOLB, TOP2a, DPOLA, DNMT, RNA, TYSY and RIR gene in the Autodock tool, and their score values were determined. A huge amount of drugs used to cure these mutant proteins and enzymes were analyzed by the Mala Cards database, available at www.malacards.org.

Comparing the side effects and performing the repositioning
With the use of the www.drugs.com website, the side effects of the Alendronate and Cytarabine were compared with those of the drugs used to cure mutant genes and enzymes. Drug repurposing includes the distinctive proof of existing compounds approved for utilization in various diseases, having a mechanism of activity that shows potential sickness amendments [5]. It is suggested that Alendronate and Cytarabine can be repositioned to use as drugs in several carcinomas and diseases. The chemical structures of Alendronate and Cytarabine are shown in Fig. 1.

![Chemical structures of Alendronate and Cytarabine](image.jpg)

**Fig. 1 Chemical structures of the:**

a) Alendronate compound, used to cure osteoporosis;

b) Cytarabine compound, used to cure chronic lymphocytic leukemia.
There is a need to create and access additional compelling pharmacological medicines. Drug repositioning offers an energizing likelihood to repurpose existing approved medicines for utilization with the benefit of giving a much faster way of treatment to the ailments than through novel drug revelation approaches [29].

Results
The interactions of drugs with various targets can presumably achieve antagonistic side effects or intentional treatments. The interactions predictions correspond to the associated expectations in an exceeding network of drug-target interactions, demonstrating similar aspects among the drugs and the targets [9]. The drug-target interactions were predicted within the style of network where the blue circles demonstrate the targets, and the red circles display the drug, while the arcs between a drug and a target represent their interaction. Dark gray arcs show a strong interaction between a drug and a target protein. Alendronate demonstrated strong interaction with FPPS, Hydroxylapatite, PTPRS, PTPRE, PTN4, and GGPPS gene. The bipartite network of Alendronate with targets and their interactions is shown in Figs. 2a and 2b.

![Alendronate interaction network](image_url)

Fig. 2 Alendronate: a) targets interaction network; b) interaction confidence with targets
Cytarabine demonstrated strong interactions with the DNA and the DPOLB gene. The bipartite network of Cytarabine with targets and their interactions ratio is shown in Figs. 3a and 3b.

![Diagram of Cytarabine interactions](image)

**Fig. 3 Cytarabine:** a) targets interactions network; b) interactions confidence with targets.

Drug interaction with a target refers to the reaction of a drug towards a target once they are regulated in a quick session; the response of a drug to a target is either an increase or a decrease in intensity [21]. The confidence score values obtained by Alendronate interacting with FPPS, Hydroxylapatite, PTPRS, PTPRE, PTN4 and GGPPS gene were 100. Confidence values obtained by the interaction of Cytarabine with DNA, DPOLB, were 100 and that of TOP2a, POLa, RNA, TYSY, DNMT and RIR gene were 20, 17, 14 and 13.

Molecular docking is the procedure of fixing a ligand inside the active site of a receptor and involves scanning for the low-energy binding modes. The scoring functions in the docking can help a docking system to productively investigate the binding space of a ligand. Thus, it is in charge of assessing the binding affinity once the right binding pose is identified [15, 26].

When Alendronate docked with FPPS, PTPRS, PTPRE, PTN4, and GGPPS gene, the interacting residues were ASP107, ASP174, ALA178, ASP710, LYS194, LYS198, ARG536, TYR388, THR385, ARG127, ARG136, GLU713, ASP882, PHE35, HIS57, PHE156 and LYS200. The ASP, ARG and LYS were common interacting residues in every docked
complex. When Cytarabine docked with DPOLB, TOP2A, DPOLA, DNMT, TYSY and RIR gene, the interacting residues were GLY135, ASN133, G7, ASP374, GLU379, LYS321, SER1189, LYS1137, G206, C316, ARG690, LEU198, MET179, ALA181, TYR230, TRP139, LYS472, ALA444, SER449 and GLN475. The common interacting residues were LYS, ASP and TYR. It was observed that ASP and LYS were common in both docked complexes of Alendronate and Cytarabine. Mostly, phi and sigma bonding were observed in all docked complexes. The docked results of Alendronate and Cytarabine are shown in Figs. 4 and 5.

**Fig. 4 Docked results of Alendronate with different genes:**
- a) FPPS; b) HAP; c) PTPRS; d) PTPRE; e) PTN4; f) GGPPS.

**Fig. 5 Docked results of Cytarabine with different genes:**
- a) DPOLB; b) TOP2a; c) DPOLA; d) DNMT; e) TYSY; f) RIR.
Both Alendronate and Cytarabine best fit in the pockets of all the proteins and do not leave the complex, which indicates stability of docked results. Basically, docking allows the researchers to monitor a database of compounds and predict the robust inhibitors in the light of various scoring functions [20]. The score ratio of all the docked complexes was larger, which demonstrated better docking results. On the bases of these docked results, it is suggested that both Alendronate and Cytarabine can be repositioned to cure these mutant genes and enzymes. The drugs currently in use to cure mutations of FPPS, Hydroxylapatite, PTPRS, PTPRE, PTN4 and GGPPS were checked for side effects; then, their side effects were compared with Alendronate. The drugs which displayed more side effects than Alendronate are listed in Table 1.

<table>
<thead>
<tr>
<th>Drugs name</th>
<th>Proposed actions</th>
<th>Targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avastine, Xeloda, Pemetrexed and Eloxatin</td>
<td>Involve in treatment of colorectal cancer</td>
<td>FPPS gene</td>
</tr>
<tr>
<td>Abraxane, Afinitor and Arimidex</td>
<td>Involve in treatment of breast cancer</td>
<td>Hydroxylapatite compound</td>
</tr>
<tr>
<td>Asacol, and Catapres</td>
<td>Involve in treatment of ulcerative colitis</td>
<td>PTPRS and PTPRE gene</td>
</tr>
<tr>
<td>Teniposide and Etoposide</td>
<td>Involve in treatment of glioma, pancreatic cancer and arthritis</td>
<td>PTN4 gene</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>Involve in treatment of hepatocellular carcinoma</td>
<td>GPPSS1 gene</td>
</tr>
</tbody>
</table>

The drugs currently in use to cure mutations of DNA, DPOLB, TOP2a, DPOLA, DNMT, RNA, TYSY and RIR gene were checked for side effects; then, their side effects were compared with Cytarabine. The drugs which displayed more side effects than Cytarabine are listed in Table 2.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Proposed actions</th>
<th>Targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carmustine, Doxorubicin and Platinol</td>
<td>Involve in treatment of HIV and bladder cancer</td>
<td>DNA,</td>
</tr>
<tr>
<td>Teniposide, Etoposide, Abraxane and Afinitor</td>
<td>Involve in treatment of breast cancer, leukemia and glioma</td>
<td>TOPa</td>
</tr>
<tr>
<td>Adrucil and Mutamycin</td>
<td>Involve in treatment of gastric cancer</td>
<td>DPOLB</td>
</tr>
<tr>
<td>Cladribine, Fludarabine and Cisplatin</td>
<td>Involve in treatment of osteosarcoma and mental retardation</td>
<td>DPOLA</td>
</tr>
<tr>
<td>Adderall and Retalin</td>
<td>Involve in narcolepsy</td>
<td>DNMT1</td>
</tr>
<tr>
<td>Gemistabine and Toposar</td>
<td>Involve in treatment of lung cancer</td>
<td>RIR1</td>
</tr>
<tr>
<td>Trimethoprim, Fluororacil and Adrucil</td>
<td>Involve in treatment of head and neck cancer and stomach cancer</td>
<td>TYSY</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>Involve in hepatocellular carcinoma</td>
<td>LncRNA</td>
</tr>
</tbody>
</table>

Alendronate and Cytarabine have fewer and minor side effects as compared to the above-mentioned drugs, so they can be repositioned to cure the sicknesses listed above.
Discussions
Learning about the collaborative associations between the drugs, their suggested targets and the different biological processes they can impact is vital to authorize the improvement of new clinical drugs. The exploration of drug–target interactions advances our awareness about the activities of drugs and their negative impacts on patients. Hence, their computational investigation is providing new applications to match the patients to ideal treatments and moreover, to discover new clinical indications of certified drugs [1].

Pemetrexed and Cisplatin are presently utilized as a measure in the treatment of lungs malignancy; yet, they reveal severe reactions such as vomiting, anemia, sore mouth, loose bowels and lack of sensation in hands and feet [25]. Fluorouracil chemotherapy has been in randomized clinical trials in head and neck cancers; still, its particular role is undiscovered [2]. It is also noted that Carmustine has not been verified to give remarkable improvements in the survival of patients with bladder tumors and HIV, who are treated with it [10].

Teniposide and Etoposide are specifically self-motivated towards hematological tumors; yet, they present poor action towards solid tumors. They harm the DNA by cooperation with TOPa and form complexes that prevent the DNA repair [28]. Sorafinib is a tyrosine kinase inhibitor that targets two varied signaling pathways, particularly, the vascular endothelial growth factor (VEGF) and the platelet-derived growth factor (PDGF). The use of Sorafinib includes hypertension, weariness, diarrhea, mucositis and a few disjoint symptoms [32].

Alendronate is one of the best and most widely studied bisphosphonates in the treatment of osteoporosis. The vertebral fractures among women are treated with the Alendronate; moreover, it lessened the danger of short stature. Alendronate diminishes the number of fractures. Alendronate has been demonstrated to be powerful at expanding bone mineral density (BMD) of the spine and aggregate hip. Furthermore, it decreases vertebral cracks in patients in long-term glucocorticoid treatment [24].

Cytarabine acts as a valuable medication in the treatment of chronic lymphocytic leukemia. Because of Cytarabine utilization, Beta-side effects are resolved, lymphadenopathy vanishes, and thrombocytopenia is essentially decreased. The patients become free of these side effects on a dose of 1500 mg Cytarabine every day for a fourteen-day cycle [3].

The repositioning of Alendronate and Cytarabine will be fruitful to overthrow the effects of carcinomas and hereditary conditions, as both these drugs have fewer side effects than the medicines usually available as healthier treatment of disorders.

Conclusion
In this study, Alendronate and Cytarabine were considered, and their interactions with other off-targeted proteins were analyzed. The Alendronate demonstrated strong interaction with FPPS, Hydroxylapatite, PTPRS, PTPRE, PTN4 and GGPPS, and Cytarabine with DNA and DPOLb. The side effects of Alendronate and Cytarabine were compared with those of several drugs mentioned in the tables, and it was concluded that Alendronate and Cytarabine have fewer side effects and demonstrate better score values on interaction than the other drugs. In the docked complexes of Alendronate and Cytarabine it was observed that ASP and LYS were common interacting residues.

On the bases of interactions and docking, it is suggested that both the Alendronate and Cytarabine can be repositioned to cure the mutations FPPS, Hydroxylapatite, PTPRS, PTPRE,
PTN4, GGPPS, DNA, DPOLB, TOP2a, POLa, RNA, DNMT, TYSY and RIR gene. In future prospects, this research work can be further utilized as a part of clinical trials to test its adequacy and suitability.

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