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

Anum Munir[1*], Shumaila Azam[1, 2], Sahar Fazal[2], Zanib Khan[1], Azhar Mehmood[1]

[1]*Department of Bioinformatics*
Government Post Graduate College Mandian
Abbottabad 22010, Pakistan
E-mails: anummunir786@yahoo.com, shumailaazam@hotmail.com, zain_jadoon@hotmail.com, azharmehmood35@yahoo.com

[2]*Department of Bioinformatics and Biosciences*
Capital University of Sciences and Technology
Islamabad 44000, Pakistan
E-mail: sahar@cust.edu.pk

[*]*Corresponding author*

Received: January 14, 2016 **Accepted: July 19, 2016**

Published: September 30, 2016

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 (TOPa) is responsible for DNA replication; over-expression of this gene results in breast cancer [7]. DNA polymerase alpha (POLa) 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

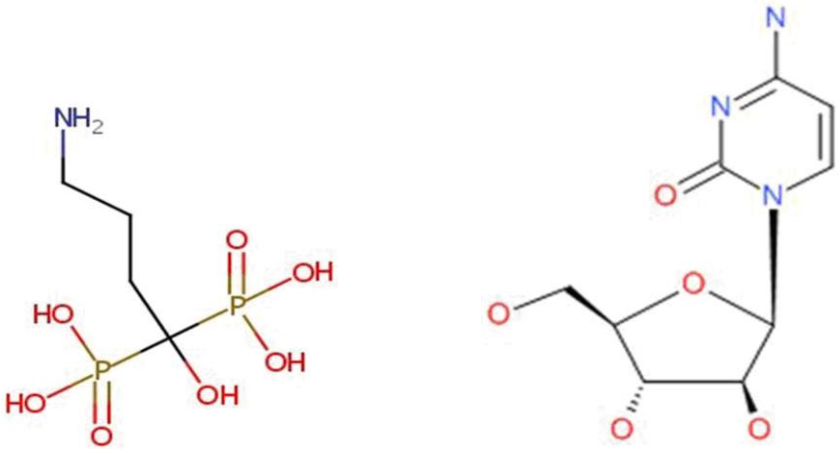
Their interactions with other off-target proteins were predicted by using the Balestra web server, available at http://balestra.csb.pitt.edu/. The drugs were repositioned to be used in several cancers as well as some other diseases. The ADMET properties and toxicity values of Alendronate and Cytarabine 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.



a) b)

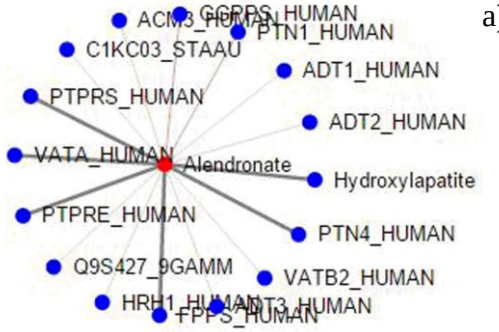
Fig. 1 Chemical structures of the:
a) Alendronate compound, used to cure osteopoeosis;
b) Cytarabine compound, used to cure chronic lymphocytic leukemia.

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.

a)



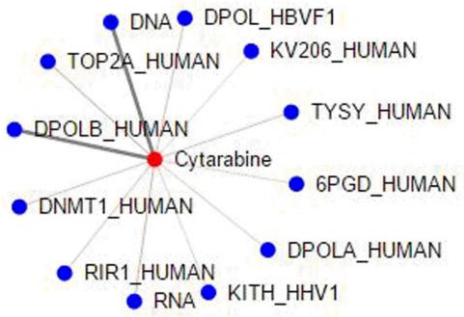
b)

Status	Name	Confidence	Strength
Known	Farnesyl pyrophosphate synthase (FPPS_HUMAN)	100.0%	
Known	Hydroxylapatite	100.0%	
Known	Receptor-type tyrosine-protein phosphatase S (PTPRS_HUMAN)	100.0%	
Known	Receptor-type tyrosine-protein phosphatase epsilon (PTPRE_HUMAN)	100.0%	
Known	Tyrosine-protein phosphatase non-receptor type 4 (PTN4_HUMAN)	100.0%	
Known	V-type proton ATPase catalytic subunit A (VATA_HUMAN)	100.0%	
Predicted	Geranylgeranyl pyrophosphate synthase (GGPPS_HUMAN)	19.9%	
Predicted	Tyrosine-protein phosphatase non-receptor type 1 (PTN1_HUMAN)	19.1%	
Predicted	ADP/ATP translocase 1 (ADT1_HUMAN)	9.1%	
Predicted	ADP/ATP translocase 3 (ADT3_HUMAN)	8.6%	
Predicted	Histamine H1 receptor (HRH1_HUMAN)	7.0%	
Predicted	V-type proton ATPase subunit B, brain isoform (VATB2_HUMAN)	6.3%	
Predicted	Muscarinic acetylcholine receptor M3 (ACM3_HUMAN)	6.3%	
Predicted	Protein-tyrosine-phosphatase (Q9S427_9GAMM)	6.3%	
Predicted	ADP/ATP translocase 2 (ADT2_HUMAN)	6.0%	
Predicted	Penicillin binding protein 2a (C1KC03_STAAU)	5.9%	

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.

a)



b)

Status	Name	Confidence	Strength
Known	DNA	100.0%	
Known	DNA polymerase beta (DPOLB_HUMAN)	100.0%	
Predicted	DNA topoisomerase 2-alpha (TOP2A_HUMAN)	20.1%	
Predicted	RNA	17.2%	
Predicted	Ribonucleoside-diphosphate reductase large subunit (RIR1_HUMAN)	14.4%	
Predicted	Protein P (DPOL_HBVF1)	14.1%	
Predicted	Thymidylate synthase (TYSY_HUMAN)	14.0%	
Predicted	DNA (cytosine-5)-methyltransferase 1 (DNMT1_HUMAN)	13.1%	
Predicted	DNA polymerase alpha catalytic subunit (DPOLA_HUMAN)	12.8%	
Predicted	Thymidine kinase (KITH_HHV1)	9.8%	
Predicted	Ig kappa chain V-II region RPMI 6410 (KV206_HUMAN)	9.4%	
Predicted	6-phosphogluconate dehydrogenase, decarboxylating (6PGD_HUMAN)	9.4%	

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.

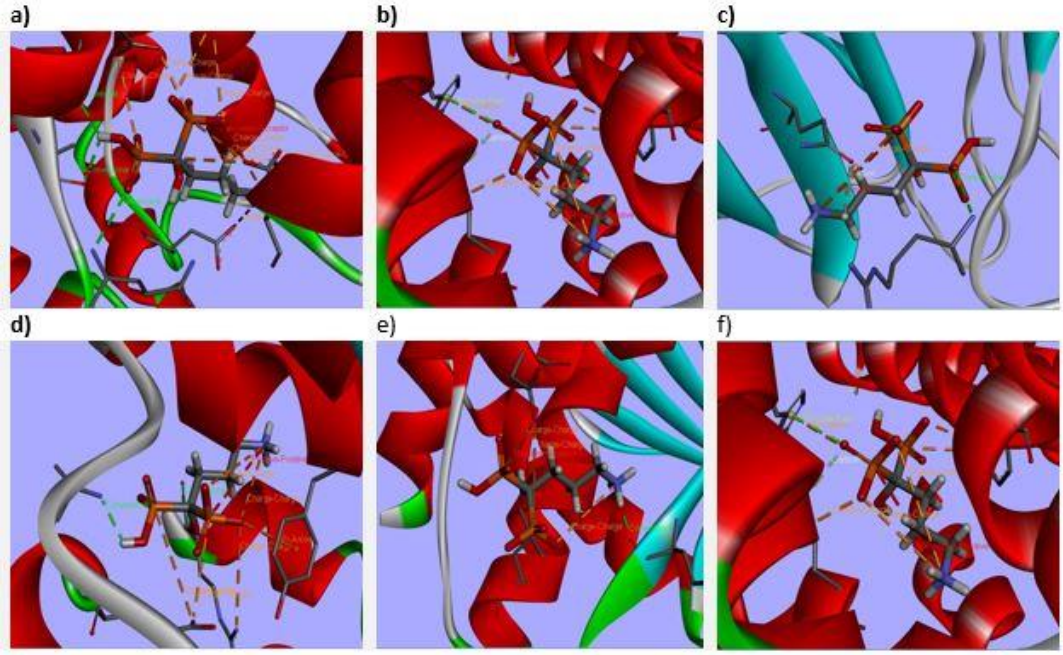


Fig. 4 Docked results of Alendronate with different genes:
a) FPPS; b) HAP; c) PTPRS; d) PTPRE; e) PTN4; f) GGPPS.

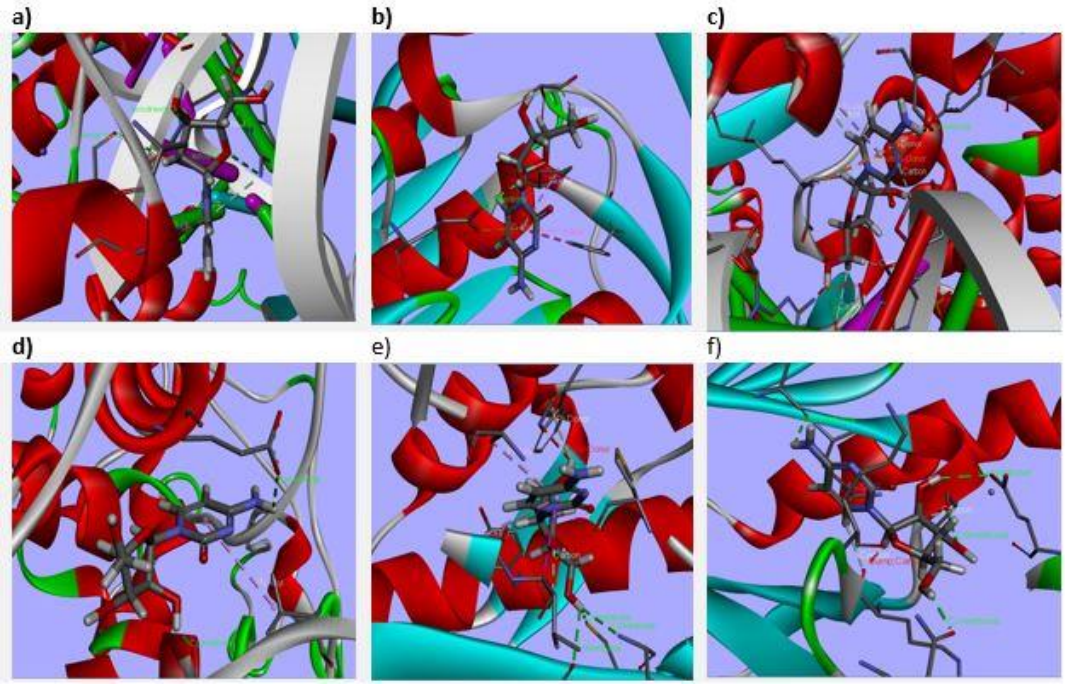


Fig. 5 Docked results of Cytarabine with different genes:
a) DPOLB; b) TOP2a; c) DPOLa; d) DNMT; e) TYSY; f) RIR.

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 1. List of drugs which were compared with the side effects of Alendronate

Drugs name	**Proposed actions**	**Targets**
Avastine, Xeloda, Pemetrexed and Eloxatin	Involve in treatment of colorectal cancer	FPPS gene
Abraxane, Afinitor and Arimidex	Involve in treatment of breast cancer	Hydroxylapatite compound
Asacol, and Catapres	Involve in treatment of ulcerative colitis	PTPRS and PTPRE gene
Teniposide and Etoposide	Involve in treatment of glioma, pancreatic cancer and arthritis	PTN4 gene
Sorafinib	Involve in treatment of hepatocellular carcinoma	GPPSS1 gene

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 2. List of drugs which were compared with the side effects of Cytarabine

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

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 presents 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 and genes 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.

Acknowledgements

This research exploration work is unique and has not been submitted in any journal yet. None of the authors has challenging conflicts of interest. Authors are grateful to Professor Shakeel Ahmed Mufti, Professor Noor-ud-din, Department of Bioinformatics and Bioinformatics Research Club of Government Post Graduate College Mandian Abbottabad for providing the platform to conduct the research.

References

1. Azuaje F. (2013). Drug Interaction Networks: An Introduction to Translational and Clinical Applications, Cardiovascular Research, 97(4), 631-641.
2. Blanchard P., J. Bourhis, B. Lacas, M. R. Posner, J. B. Vermorken, J. J. Cruz Hernandez, A. Bourredjem, G. Calais, A. Paccagnella, R. Hitt, J. P. Pignon on behalf of the Meta-Analysis of Chemotherapy in Head and Neck Cancer, Induction Project, Collaborative Group (2013). Taxane-cisplatin-fluorouracil as Induction Chemotherapy in Locally Advanced Head and Neck Cancers: An Individual Patient Data Meta-Analysis of the Meta-Analysis of Chemotherapy in Head and Neck Cancer Group, Journal of Clinical Oncology, 31(23), 2854-2860.
3. Braess J., W. Kern, M. Unterhalt, C. C. Kaufmann, B. Ramsauer, M. Schüssler, A. Kaeser-Fröhlich, W. Hiddemann, E. Schleyer (1996). Response to Cytarabine Ocfosfate (YNK01) in a Patient with Chronic Lymphocytic Leukemia Refractory to Treatment with Chlorambucil/prednisone, Fludarabine, and Prednimustine/mitoxantrone, Annals of Hematology, 73(4), 201-204.
4. Cheng F., C. Liu, J. Jiang, W. Lu, W. Li, G. Liu, W. Zhou, J. Huang, Y. Tang (2012). Prediction of Drug-target Interactions and Drug Repositioning via Network-based Inference, PLoS Computational Biology, 8(5), e1002503.
5. Corbett A., G. Williams, C. Ballard (2013). Drug Repositioning: An Opportunity to Develop Novel Treatments for Alzheimer's Disease, Pharmaceuticals, 6(10), 1304-1321.
6. Davidson J. D., L. Ma, M. Flagella, S. Geeganage, L. M. Gelbert, C. A. Slapak (2004). An Increase in the Expression of Ribonucleotide Reductase Large Subunit 1 Is Associated with Gemcitabine Resistance in Non-small Cell Lung Cancer Cell Lines, Cancer Research, 64(11), 3761-3766.
7. Depowski P. L., S. I. Rosenthal, T. P. Brien, S. Stylos, R. L. Johnson, J. S. Ross (2000). Topoisomerase IIα Expression in Breast Cancer: Correlation with Outcome Variables, Modern Pathology, 13(5), 542-547.
8. Eichhorst B., M. Dreyling, T. Robak, E. Montserrat, M. Hallek on behalf of the ESMO Guidelines Working Group (2011). Chronic Lymphocytic Leukemia: ESMO Clinical Practice Guidelines for Diagnosis, Treatment and Follow-up, Annals of Oncology, 22(Supp. 6), vi50-vi54.
9. Fakhraei S., L. Raschid, L. Getoor (2013). Drug-target Interaction Prediction for dRug Repurposing with Probabilistic Similarity Logic, Proceedings of the 12th International Workshop on Data Mining in Bioinformatics, 10-17.
10. Garside R., M. Pitt, R. Anderson, G. Rogers, M. Dyer, S. Mealing, M. Somerville, A. Price, K. Stein (2007). The Effectiveness and Cost-effectiveness of Carmustine Implants and Temozolomide for the Treatment of Newly Diagnosed High-grade Glioma: A Systematic Review and Economic Evaluation, Health Technology Assessment, 11(45), iii-iv, ix-221.

11. Ishikawa C., T. Matsuda, T. Okudaira, M. Tomita, H. Kawakami, Y. Tanaka, M. Masuda, K. Ohshiro, T. Ohta, N. Mori (2007). Bisphosphonate Incadronate Inhibits Growth of Human T-cell Leukaemia Virus Type I-infected T-cell Lines and Primary Adult T-cell Leukaemia Cells by Interfering with the Mevalonate Pathway, British Journal of Haematology, 136(3), 424-432.
12. Jin G., S. T. C. Wong (2014). Toward Better Drug Repositioning: Prioritizing and Integrating Existing Methods into Efficient Pipelines, Drug Discovery Today, 19(5), 637-644.
13. Karahalil B., N. A. Kocabas, T. Özçelik (2006). DNA Repair Gene Polymorphisms and Bladder Cancer Susceptibility in a Turkish Population, Anticancer Research, 26(6C), 4955-4958.
14. Li G., H. Zhang, X. Wan, X. Yang, C. Zhu, A. Wang, L. He, R. Miao, S. Chen, H. Zhao (2014). Long Noncoding RNA Plays a Key Role in Metastasis and Prognosis of Hepatocellular Carcinoma, BioMed Research International, 2014, Article ID 780521.
15. Li Z., X. Wang, K. Li, J. Gu, L. Kang (2014.). Molecular Docking Improvement: Coefficient Adaptive Genetic Algorithms for Multiple Scoring Functions, International Journal Bioautomantion, 18(1), 5-14.
16. Loeb L. A., R. J. Monnat (2008). DNA Polymerases and Human Disease, Nature Reviews Genetics, 9(8), 594-604.
17. Meena R., K. K. Kesari, M. Rani, R. Paulraj (2012). Effects of Hydroxyapatite Nanoparticles on Proliferation and Apoptosis of Human Breast Cancer Cells (MCF-7), Journal of Nanoparticle Research, 14(2), 14:712.
18. Muise A. M., T. Walters, E. Wine, A. M. Griffiths, D. Turner, R. H. Duerr, M. D. Regueiro, B.-Y. Ngan, W. Xu, P. M. Sherman, M. S. Silverberg, D. Rotin (2007). Protein-tyrosine Phosphatase Sigma Is Associated with Ulcerative Colitis, Current Biology, 17(14), 1212-1218.
19. Munir A., S. Azam, S. Fazal (2015). Repositioning of Methotrexate to Cure Mutations of DYR, TYMS and PURA Gene, Bleomycin in the Treatment of Mutant DNA, TOPa, POLa, and RIR Genes, Merit Research Journal of Medicine and Medical Sciences, 3(7), 249-255.
20. Munir A., S. Khan, S. Azam (2015). Computational Drug ZINPIP-analog an Ultimate Solution to Cure Conserved Domains of Mutant EGFR, ALK and BRAF Proteins in NSCLC, International Current Pharmaceutical Journal, 4(7), 396-401.
21. Nidhi S. (2012). Concept of Drug Interaction, International Research Journal of Pharmacy, 3(7), 120-122.
22. Notarnicola M., C. Messa, M. G. Caruso (2012). A Significant Role of Lipogenic Enzymes in Colorectal Cancer, Anticancer Research, 32(7), 2585-2590.
23. Papaioannou A., S. Morin, A. M. Cheung, S. Atkinson, J. P. Brown, S. Feldman, D. A. Hanley, A. Hodsman, S. A. Jamal, R. G. Josse, S. M. Kaiser, B. Kvern, K. Siminoski, W. D. Leslie; for the Scientific Advisory Council of Osteoporosis Canada (2010). Clinical Practice Guidelines for the Diagnosis and Management of Osteoporosis in Canada: Background and Technical Report. http://www.osteoporosis.ca/multimedia/pdf/Osteoporosis_Guidelines_2010_Background_And_Technical_Report.pdf (Last accessed August 15, 2016)
24. Prinsloo P. J. J., D. J. Hosking (2006). Alendronate Sodium in the Management of Osteoporosis, Therapeutics and Clinical Risk Management, 2(3), 235-249.
25. Pufe T., M. Bartscher, W. Petersen, B. Tillmann, R. Mentlein (2003). Expression of Pleiotrophin, an Embryonic Growth and Differentiation Factor, in Rheumatoid Arthritis, Arthritis & Rheumatism, 48(3), 660-667.
26. Singh A., S. Kumari, T. K. Pal (2015). *In silico* Analysis for Laccase-mediated

Bioremediation of the Emerging Pharmaceutical Pollutants, International Journal Bioautomation, 19(4), 423-432.
27. Tan X., H. Wang, G. Luo, S. Ren, W. Li, J. Cui, H. S. Gill, S. W. Fu, Y. Lu (2015). Clinical Significance of a Point Mutation in DNA Polymerase Beta (POLB) Gene in Gastric Cancer, International Journal of Biological Sciences, 11(2), 144-155.
28. Thakur D. S. (2011). Topoisomerase II Inhibitors in Cancer Treatment, International Journal of Pharmaceutical Sciences and Nanotechnology, 3(4), 1173-1181.
29. What Is Osteoporosis?, http://www.niams.nih.gov/health_info/Bone/Osteoporosis/osteoporosis_ff.pdf (Last accessed August 15, 2016)
30. Winkelmann J., L. Lin, B. Schormair, B. R. Kornum, J. Faraco, G. Plazzi, A. Melberg, F. Cornelio, A. E. Urban, F. Pizza, F. Poli, F. Grubert, T. Wieland, E. Graf, J. Hallmayer, T. M. Strom, E. Mignot (2012). Mutations in DNMT1 Cause Autosomal Dominant Cerebellar Ataxia, Deafness and Narcolepsy, Human Molecular Genetics, 21(10), 2205-2210.
31. Yang L., J. Chen, L. Shi, M. P. Hudock, K. Wang, L. He (2010). Identifying Unexpected Therapeutic Targets via Chemical-protein Interactome, PLoS ONE, 5(3), e9568.
32. Zhang Z., Q. Shi, E. M. Sturgis, M. R. Spitz, W. K. Hong, Q. Wei (2004). Thymidylate Synthase 5′-and 3′-untranslated Region Polymorphisms Associated with Risk and Progression of Squamous Cell Carcinoma of the Head and Neck, Clinical Cancer Research, 10(23), 7903-7910.

Anum Munir, B.Sc. (Hons)
E-mail: anummunir786@yahoo.com

Anum Munir has done B.Sc. (Hons) in Bioinformatics at the Government Post Graduate College Mandian (GPGCM), Abbottabad, Pakistan. She works as a bioinformatics researcher and is an active participant in certain ongoing research projects. She is a dedicated member of the Bioinformatics research club GPGCM. She has command over many bioinformatics tools and software used in drug designing and in other activities related to bioinformatics. Her major interests and expertise are in drug discovery, drug designing, drug repositioning, drug repurposing, pharmacophore modeling, statistical analysis, phylogenetic analysis, protein structure and function prediction, PBPK modeling, system biology, vaccine designing and pharmacognostic studies. She works with various programming languages such as MATLAB, Java, C++ and Python. Up till now she has more than 6 publications and a few are submitted in reputable journals.

Shumaila Azam, M.Sc. (Hons)
E-mail: shumailaazam@hotmail.com

Shumaila Azam has done M.Sc. (Hons) in Bioinformatics at the Capital University of Sciences and Technology (CUST), Islamabad, Pakistan. She has completed her B.Sc. (Hons) at the same university. She is working as a lecturer in bioinformatics at the GPGCM, Abbottabad. She is a dedicated member of the research group of CUST and a team leader of the Bioinformatics research club GPGCM. She has command over many bioinformatics tools and software. Her major interests include drug discovery, drug designing, drug repositioning, phylogenetic analysis,

pathways and networks determination, system biology, system medicine, NGS analysis, gene mining, big data analysis and microarray data analysis. She is participating in many ongoing research projects. She works with various programming languages such as R, MATLAB, Java, C++ Perl Python and Oracle. She has 4 publications up till now and a few are submitted.

Assoc. Prof. Sahar Fazal, Ph.D.
E-mail sahar@cust.edu.pk

Dr. Sahar Fazal has completed her Post-Doctoral degree in Biochemistry and Molecular Genetics at the University of Sussex, Brighton, UK and her Ph.D. in Applied Chemistry at the South China Agriculture University. She is currently working as an Associate Professor at the CUST, Islamabad. She is a dedicated member of the research group of CUST. Her major interests include bioinformatics (phylogenetic, protein interactions, modeling, pathways), resistance management, molecular entomology, microbiology and genetics. Up till now she has had 14 publications and has won many awards in different areas of her studies.

Zanib Khan, M.Sc. (Hons)
E-mail: zain_jadoon@hotmail.com

Zanib Khan has completed her B.Sc. (Hons) in Microbiology at the Hazara University and got her M.Sc. (Hons) in Biotechnology from Comsat institute Abbottabad. She is also working as a lecturer of bioinformatics and microbiology at the GPGCM, Abbottabad. She has command over many techniques in biotechnology as well as techniques of microbiology. Her major interests include extraction of antibiotics from soil dwelling microbes, epidemics of infectious diseases, molecular characterization of different strains, and identification of bacterial strains.

Assoc. Prof. Azhar Mehmood, Ph.D.
E-mail: azharmehmood35@yahoo.com

Azhar Mehmood has a Ph.D. in Phyto-sociology and has also completed his M.Phil. in Tissue Culture. He is working as an Associate Professor and as a Head of the Department of Bioinformatics at the GPGCM, Abbottabad. He is also a team member of the Bioinformatics research club GPGCM. He has a teaching experience of 18 years and his major interests are phyto-sociology, genetics, and tissue culture.