Original Research Article

Repositioning of Methotrexate to Cure Mutations of DYR, TYMS and PURA Gene, Bleomycin in the Treatment of Mutant DNA, TOPa, POLa, and RIR Genes

Anum Munir[1*], Shumaila Azam[1,2] and Sahar Fazal[2]

Abstract

[1]Department of Bioinformatics Government Post Graduate College Mandian, Abbottabad 22010, Pakistan.

[2]Department of Bioinformatics Muhammad Ali Jinnah University Islamabad 44000, Pakistan. Post Doc, Professor.

Corresponding Author's E-mail: anummunir786@yahoo.com Tel.: +923348958178

Osteosarcoma occurs because of germ line transformations of P53 gene and RB gene. At present doxorubicin, cisplatin and Methotrexate are viewed as dynamic drugs to cure osteosarcoma. Lymphoma grows because of neoplastic transformations of CD20, CD40, LMP gene, and B cells. Currently rituximab and Bleomycin are utilized as monoclonal antibodies against this ailment. Drug repositioning is new emerging phenomenon of reusing old drugs, protecting retired drugs and developing licenses to make lives easy. The primary objective of study was repositioning of methotrexate and Bleomycin to use in other diseases also. We found out interactions of both these drugs with several off targets, methotrexate showed strong interactions with DYR gene, Bleomycin demonstrated strong interactions with DNA, DNL1 and DNL3. After screening large amount of drugs which are used to cure mutations of those off target genes and proteins, compared their side effects and suggested that methotrexate and Bleomycin have fewer side effects as compared to other drugs which are used in same interacting targets. Both methotrexate and Bleomycin can be reposition to cure certain carcinomas and other diseases.

**Keywords:** Bleomycin, Interactions, Lymphoma, Methotrexate, Osteosar-coma, Repositioning

INTRODUCTION

Osteosarcoma, the most well-known solid malignancy of bone results from the development of malignant mesenchyme cells thus results in the formation of osteoid in bones. Osteosarcoma occurs because of germ line transformations of P53 gene and RB gene, generally has great impact on long bones and areas around knee and forearms. Treatments involve surgery, radiotherapy and systemic therapy. At present doxorubicin, cisplatin and methotrexate are viewed as dynamic drugs to cure osteosarcoma (Ritter and Bielack, 2010; Ogata et al., 2011; Geller and Gorlick, 2010) Lymphoma is heterogeneous group of malignancies occurs in lymph nodes and lymphatic system. Lymphoma grows because of neoplastic transformations of CD20, CD40, LMP gene, and B cells. Currently rituximab and Bleomycin are utilized as monoclonal antibodies against this ailment

(Matasar and Word, 2012; Kuppers et al., 2012)

Anemia happens because of folate insufficiency and brings about few malignancies. DYR and DHFR gene are responsible for maintaining folate homeostasis. Mutations in DHFR gene causes anemia (Cario et al., 2011). Head and neck carcinoma incorporate malignancy of oral cavity, pharynx, larynx and mouth. TYMS gene is responsible for the regulation of folate metabolism. Mutation in TYMS gene causes head and neck cancer (Zhang et al., 2004). Transformation in PURA gene results in micro deletion syndrome characterized by neurodevelopment delay, epilepsy and hypotonia (Lalani et al., 2014).

Oxidative DNA damage is incited by oxygen elements that outcome in the development of bladder cancer (Karahil et al., 2006). DNA Topoisomerase alpha (TOPa)
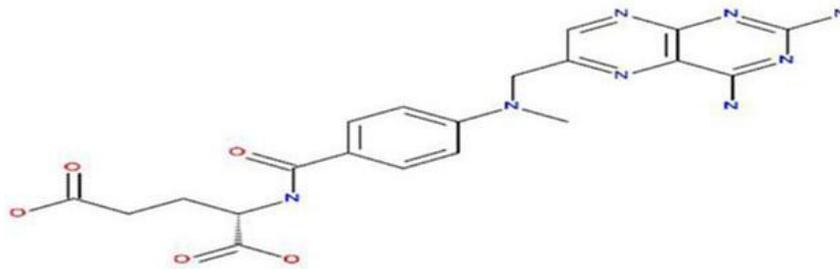
Figure 1. Chemical structure of Methotrexate compound used to cure osteosarcoma
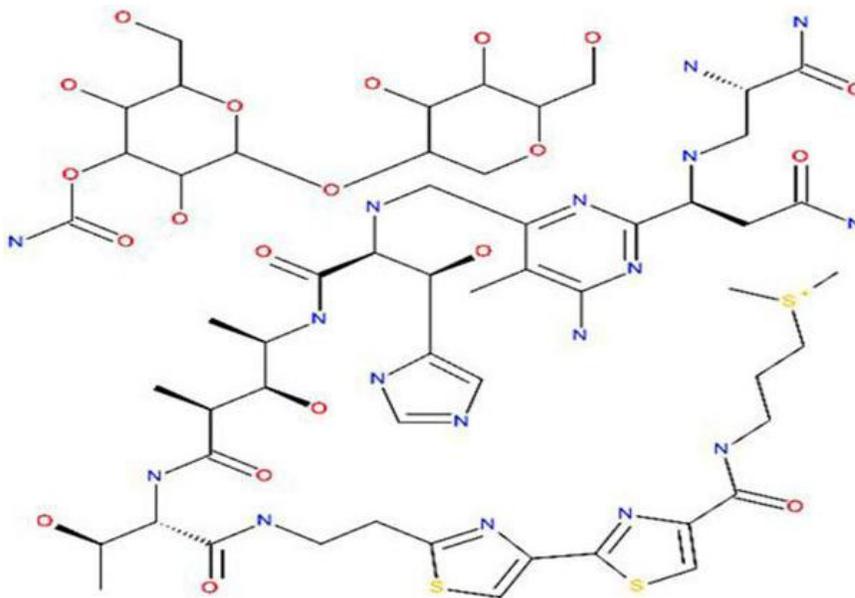
**Figure 2.** Chemical structure of Bleomycin compound used to cure lymphoma

is responsible for DNA replication, over expression of this gene results in breast cancer (Depowski et al., 2000). DNA polymerase alpha (POLa) exhibit in distinct five classes and performs the role in DNA replication and repair. The substantial transformation of DNA polymerase alpha results in adenocarcinoma of colon and ophthalmoplegia (Loeb and Monnat, 2008). Ribonucleotide reductase large subunits (RIR) are needed for DNA polymerization and repair, over expression of RRM results in non-small cell lung cancer and pancreatic cancer (Davidson et al., 2004).

Drug molecules not only influence their proposed protein targets but also to other targets as well, drug protein interactions prompt the disclosure of new therapeutic targets and pathways. Drug repositioning is new emerging phenomenon of reusing old drugs, protecting retired drugs and developing licenses to make lives easy. Docking one drug to a multi-protein set has been utilized as a sensible methodology. Drug target association is the premise of drug disclosure and configuration but is time consuming and costly process,

the only alternative solution to this problem is the use of computational methods to predict drug-target interactions and perform repositioning of drugs (Jin and Wong, 2014; Yang et al., 2010; Cheng et al., 2012).

MATERIAL AND METHOD

After screening large amount of drugs which are used in osteosarcoma and lymphoma, we selected methotrexate that is used to cure osteosarcoma and Bleomycin used to cure lymphoma.We predicted their interactions with other off target proteins and repositioned them to use as anti-cancerous drugs in other diseases. We calculated ADMET properties and toxicity values of methotrexate and Bleomycin, docked methotrexate with DYR, TYMS and PURA gene, Bleomycin with TYMS gene, DNA, TOPa, POLa, and RIR1 enzyme and determined their score values. We analyzed huge measure of drugs used to cure these mutant genes and enzymes. With the use of drugs.com website we compared the side effects of
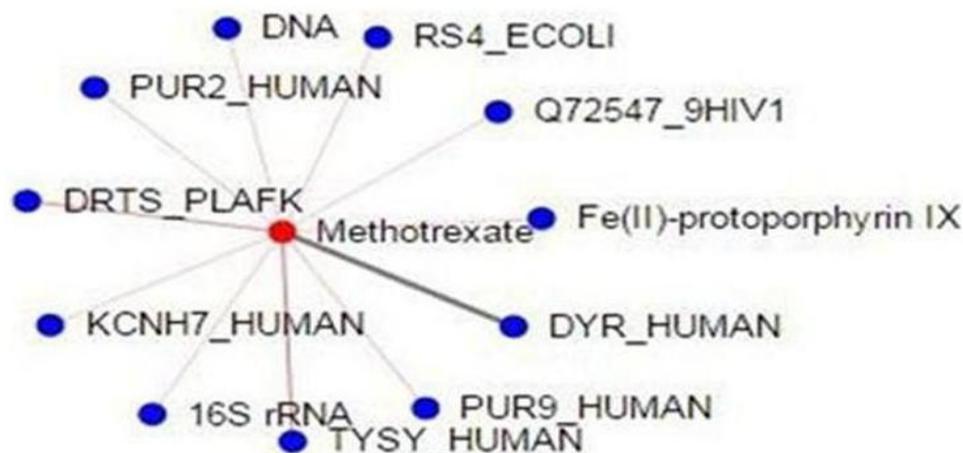
Figure 3a. Methotrexate targets interaction network

Status	Name	Confidence	Strength
Known	Dihydrofolate reductase (DYR_HUMAN)	100.0%	
Predicted	Thymidylate synthase (TYSY_HUMAN)	24.9%	
Predicted	Bifunctional dihydrofolate reductase-thymidylate synthase (DRTS_PLAFK)	21.6%	
Predicted	Trifunctional purine biosynthetic protein adenosine-3 (PUR2_HUMAN)	12.4%	
Predicted	Bifunctional purine biosynthesis protein PURH (PUR9_HUMAN)	12.2%	
Predicted	16S rRNA	5.2%	
Predicted	DNA	4.5%	
Predicted	Fe(II)-protoporphyrin IX	4.4%	
Predicted	Reverse transcriptase/RNaseH (Q72547_9HIV1)	4.2%	
Predicted	Potassium voltage-gated channel subfamily H member 7 (KCNH7_HUMAN)	3.9%	
Predicted	30S ribosomal protein S4 (RS4_ECOLI)	3.8%	

Figure 3b. Interaction confidence of methotrexate with targets

methotrexate and Bleomycin with those drugs. Drug repurposing includes the distinguishing proof of existing compounds that are authorized for utilization for different diseases yet, which have mechanism of activity that show potential illness change (Corbett et al., 2013). We suggested that the methotrexate and Bleomycin can be reposition to use as drugs in several carcinomas and diseases. The chemical structures of methotrexate and Bleomycin are shown in Figure 1 and Figure 2.

There is a critical need to create and access more compelling pharmacological medicines. Drug repositioning offers an energizing chance to repurpose existing authorized drugs for utilization with the advantage of giving a much quicker course to the facility than through novel drug disclosure approaches (Corbett et al., 2013).

RESULTS

The interactions of drugs with various targets can possibly bring about antagonistic side effects or intentional treatments. The interactions predictions correspond to the connection expectations in a network of drug-target interactions, represents set of similarities among drugs and targets (Fakhraei et al., 2013). The drug-target interactions were predicted in the form of network where the blue circles represent the targets and red circles represents the drug, links between drug and target indicate their interaction. Dark gray link indicates strong interaction between drug and target protein. Methotrexate represented strong interaction with DYR gene. The bipartite network of methotrexate with targets and their interactions are shown in Figure 3a and 3b.
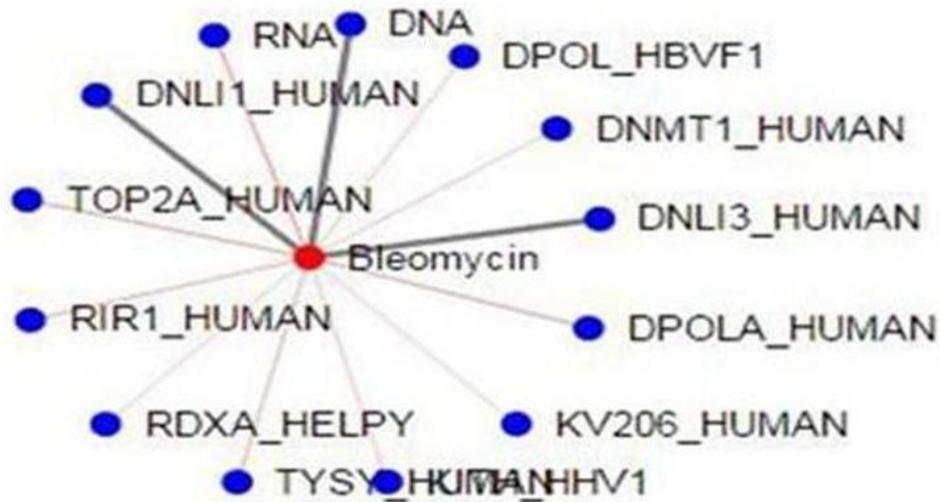
Figure 4a. Bleomycin -targets interactions network

Status	Name	Confidence	Strength
Known	DNA	100.0%	
Known	DNA ligase 1 (DNLI1_HUMAN)	100.0%	
Known	DNA ligase 3 (DNLI3_HUMAN)	100.0%	
Predicted	RNA	18.9%	
Predicted	DNA topoisomerase 2-alpha (TOP2A_HUMAN)	18.3%	
Predicted	DNA polymerase alpha catalytic subunit (DPOLA_HUMAN)	17.7%	
Predicted	Ribonucleoside-diphosphate reductase large subunit (RIR1_HUMAN)	14.7%	
Predicted	Thymidylate synthase (TYSY_HUMAN)	13.9%	
Predicted	DNA (cytosine-5)-methyltransferase 1 (DNMT1_HUMAN)	10.8%	
Predicted	Thymidine kinase (KITH_HHV1)	10.3%	
Predicted	Ig kappa chain V-II region RPMI 6410 (KV206_HUMAN)	10.2%	
Predicted	Protein P (DPOL_HBVF1)	10.2%	
Predicted	Oxygen-insensitive NADPH nitroreductase (RDXA_HELPY)	9.6%	

Figure 4b. Interactions confidence of Bleomycin with targets

Bleomycin represented strong interactions withDNA, DNA ligase 1 (DNL1) and DNA ligase 3 (DNL3). The bipartite network of Bleomycin with targets and their interactions ratio are shown in Figure 4a and 4b.

Drug interaction to the target alludes to the reaction of drug towards target when they are regulated in fast session; the response of drug to target is either expanded or diminished in intensity (Nidhi, 2012). The confidence score values obtained by interacting methotrexate with DYR, TYMS, PUR2 and PUR9 were 100, 24.9, 12.4 and 12.2. Confidence values obtained by the interaction of Bleomycin with DNA, DNL1, DNL2 were 100 and that of with TOPa, POLa, RIR1, and TYMS gene were 18.3,

17.7, 14.7 and13.9

The drugs currently in use to cure mutations of DYR, TYMS, PUR2 and PUR9 were checked for side effects then their side effects were compared with methotrexate. The drugs which showed more side effects than methotrexate are listed in Table 1.

Commonly available drugs in market which are in use to cure mutations of DNA, DNL1, DNL2, TOPa POLa RIR1, and TYMS gene and were checked for side effects then their side effects were compared with Bleomycin. The drugs which showed more side effects than Bleomycin are listed in Table 2.

Methotrexate and Bleomycin have less and minor side

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

Drug name	Proposed actions	Targets
Pyremathamine	Involve in treatment of anemia, malaria and osteosarcoma	DYR gene
Trimethoprim	Involve in treatment of head and neck cancer and stomach cancer	TYMS gene
Fluorouracil	Involve in treatment of head and neck cancer and stomach cancer	TYMS gene
Pemetrexed	Involve in treatment of Alzheimer's, colorectal cancer, osteoporosis and arthritis	PUR2 and PUR9 gene

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

Drugs	Proposed actions	Targets
Carmustine	Involve in treatment of HIV and bladder cancer	DNA, DNL1 and DNL3
Teniposide	Involve in treatment of breast cancer, leukemia and glioma	TOPa
Etoposide	Involve in treatment of breast cancer, leukemia and glioma	TOPa
Cladribine	Involve in treatment of osteosarcoma and mental retardation	POLa
Fludarabine	Involve in treatment of osteosarcoma and mental retardation	POLa
Gemcitabine	Involve in treatment of lung cancer	RIR1
Trimethoprim	Involve in treatment of head and neck cancer and stomach cancer	TYMS
Fluorouracil	Involve in treatment of head and neck cancer and stomach cancer	TYMS

effects as compared to above mentioned drugs so they can be reposition to use in the treatment of above mentioned diseases.

DISCUSSIONS

Learning about the collaborations between drugs, their proposed targets and apparently different random biological procedures that they can influence is vital to empower the advancement of new clinical applications. The investigation of drug–target interactions improves our insight into the components of activities of drugs and their unfavorable impacts in patients. Thus, their computational examination is empowering new applications to match patients to ideal treatments, furthermore to discover new clinical evidences of endorsed drugs (Azuaje, 2013).

Pyremathamine is normally used to cure patients those endure intense frailty however Pyremathamine seems to be not sufficiently powerful (Mockenhaupt et al., 2001). Pemetrexed and cisplatin are presently utilized as a part of the treatment of lungs malignancy yet demonstrate extreme reactions as retching, paleness, sore mouth, loose bowels and deadness in hands and feet (Mackmillan and Cancer support, n.d.).

Fluorouracil affectation chemotherapy has been in randomized trials in loco provincially propelled head and neck diseases, its exact part is still stays to be undiscovered (Balanchard et al., 2013). It is also noted that Carmustine has not been demonstrated to give a noteworthy favorable position in survival for patients with bladder tumors and HIV when treated with it (Garside et al., 2007).

Teniposide and Etoposide are especially dynamic towards hematological tumors yet show constrained action towards solid tumors. They harm DNA by collaboration with TOPa and form complexes that prevent the mechanism of DNA repair (Thakur, 2011).

Methotrexate, due to its adequacy and securityis settled as the anchor drug for treatment of rheumatoid joint inflammation. Despite the fact that Methotrexate is commonly directed orally, Methotrexate offers more noteworthy bioavailability and may bring about less gastrointestinal deplorability and may have fewer side effects than other drugs (Keystone and Freundlich, 2014).

Bleomycin in blend chemotherapy reliably delivers 70% complete abatements from disease, a further 10% of

patients rendered infection free after surgical extraction of remaining disease, thus it results as fruitful in the satisfactory removal of certain ailments (Praveen and Chowdary, 2013).

The repositioning of methotrexate and Bleomycin will be productive to defeat the effects of carcinomas and hereditary sicknesses, as both these drugs have less symptoms than the medications generally accessible as better treatment of illnesses.

CONCLUSION

In our research work took Methotrexate and Bleomycin, performed their interactions with other off targeted proteins and genes. The methotrexate demonstrated strong interaction with DYR gene, Bleomycin with DNA, DNL1 and DNL3. We compared side effects of Methotrexate and Bleomycin with Carmustine, Pemetrexed and several other drugs and conclude that Methotrexate and Bleomycin have fewer side effects, and demonstrate better score values on interaction than these drugs.

On the bases of this conclusion we suggest that both the Methotrexate and Bleomycin can be reposition to cure the mutations of DYR, TYMS, PURA gene, and DNA, DNL1, DNL2, TOPa, POLa, and RIR1 enzymes. In future this research exploration work can be further used as a piece of clinical trials to test its sufficiency and social focal points.

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