

Repurposing of the Syk inhibitor fostamatinib using a machine learning algorithm

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Abstract. TAM (TYRO3, AXL, MERTK) receptor tyrosine kinases (RTKs) have intrinsic roles in tumor cell proliferation, migration, chemoresistance, and suppression of antitumor immunity. The overexpression of TAM RTKs is associated with poor prognosis in various types of cancer. Single-target agents of TAM RTKs have limited efficacy because of an adaptive feedback mechanism resulting from the cooperation of TAM family members. This suggests that multiple targeting of members has the potential for a more potent anticancer effect. The present study used a deep-learning based drug-target interaction (DTI) prediction model called molecule transformer-DTI (MT-DTI) to identify commercially available drugs that may inhibit the three members of TAM RTKs. The results showed that fostamatinib, a spleen tyrosine kinase (Syk) inhibitor, could inhibit the three receptor kinases of the TAM family with an $IC_{50} < 1 \mu M$. Notably, no other Syk inhibitors were predicted by the MT-DTI model. To verify this result, this study performed *in vitro* studies with various types of cancer cell lines. Consistent with the DTI results, this study observed that fostamatinib suppressed cell proliferation by inhibiting TAM RTKs, while other Syk inhibitors showed no inhibitory activity. These results suggest that fostamatinib could exhibit anticancer activity as a pan-TAM inhibitor. Taken together, these findings demonstrated that this artificial intelligence model could be effectively used for drug

repurposing and repositioning. Furthermore, by identifying its novel mechanism of action, this study confirmed the potential for fostamatinib to expand its indications as a TAM inhibitor.

Introduction

TAM receptors are a family of receptor tyrosine kinases (RTKs) consisting of TYRO3, AXL, and MERTK that are involved in cell survival, innate immunity, and immunosuppression (1-6). They are overexpressed in various types of tumor cells and immune cells and activate several downstream signaling pathways such as phosphatidylinositol 3-kinase, extracellular signal-regulated kinase, and nuclear factor kappa-light-chain-enhancer of activated B cells pathways. These downstream signaling pathways play a role in promoting the growth and metastasis of tumor cells. Therefore, activation of TAM receptors has shown the correlations with tumor progression and poor prognosis in many cancers and is also recognized as a mediator of acquired resistance to chemotherapy and targeted therapy (1,2,7). With regard to its immunomodulatory role, previous studies using a syngeneic breast cancer model showed that conditional knockout of MERTK caused an increase in tumor-infiltrating lymphocytes and inflammatory cytokines, showing that antitumor immunity could be promoted. Numerous studies suggest that genetic modification or pharmacologic inhibition of TAM receptors can act as therapeutic targets to prevent tumor progression (8-12).

Several TAM tyrosine kinase inhibitors have been developed, and some are undergoing clinical trials for cancer treatment. The first-in-class AXL inhibitor R428, is being actively investigated in several cancers including acute myeloid leukemia, non-small cell lung cancer, and melanoma (13). Another MERTK inhibitor, UNC2025, has a 40-fold higher inhibitory activity on MERTK than AXL. Mice treated with UNC2025 show a significant reduction in tumor size and increased survival compared with vehicle-treated mice in leukemia models (14,15). However, other studies have indicated that inhibition of a single TAM receptor can result in drug resistance by inducing intrinsic and adaptive compensatory mechanisms by activating other subunits. R428 showed

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an increase in the expression of MERTK protein both *in vitro* and *in vivo* in proportion to AXL inhibition, thereby reducing the efficacy of the drug. In addition, the combined targeting of MERTK and AXL showed a synergistic antitumor effect. Based on these results, it has been hypothesized that targeting multiple members of the TAM family would be a more effective cancer treatment than single-target agents (16,17).

Pan-TAM inhibitors, such as BMS-777607 and INCB081776, are under development for the treatment of cancer, but it will take some time to achieve Food and Drug Administration (FDA) approval. Despite the development of high-throughput screening technologies, both a considerable cost and a long development time are required for a drug to be approved and released to the market. Above all, a significant number of novel drug candidates have failed in preclinical or clinical trials for various reasons, such as poor pharmacokinetics, unexplained toxicity, and lack of efficacy. Therefore, drug repurposing, which repurposes existing drugs into new treatments for intractable diseases, is being proposed as one of the methods to overcome the difficulties of drug development from novel compounds. Another advantage of drug repurposing is that existing drugs have a proven safety profile, so there are relatively few concerns about safety issues. Recently, there have been many attempts in the drug repurposing area that are expected to increase the success rate of drug development using advanced *in silico* strategies (18,19). Virtual screening using machine learning and artificial intelligence (AI) provides insights into finding new anticancer drug candidates from a large-scale drug-target interaction (DTI) dataset, including BindingDB (20), PubChem (21), and ChEMBL (22). Our group has developed a new deep DTI prediction model called Molecule Transformer (MT)-DTI (23). In 2020, using MT-DTI, we predicted that atazanavir and remdesivir were drugs that could inhibit SARS-CoV-2 (24). We then searched for a drug among commercially available medications that could simultaneously target AXL, MERTK, and TYRO3, using the MT-DTI algorithm. As a result, fostamatinib, one of the spleen tyrosine kinase (Syk) inhibitors, was predicted to inhibit TAM family members effectively, and we further verified this result using several cancer cell lines. Fostamatinib has been approved as a drug for treating chronic immune thrombocytopenia (ITP) (25), but its inhibitory effect on TAM kinases has not been reported. Here, we demonstrate that the inhibitory effect on the TAM family is a unique function of fostamatinib that is not observed with other Syk inhibitors. Our results suggest not only the possibility of expanding the use of fostamatinib by revealing a new function but also suggest the possibility of identifying new drug mechanisms through AI.

Materials and methods

Prediction of bioactivity and binding affinity for TAM kinases. The Deargen MT-DTI was used for the chemical prediction to target triple TAM kinases simultaneously (23). Based on a manually curated public database, the model was trained on bioactivity data (IC_{50}/EC_{50}) and affinity data (K_d) from the ChEMBL database (22), Drug Target Common database (26), and BindingDB database (27). MT-DTI uses a GCN (28) algorithm to extract molecular features and a ProtBert (29)

algorithm to extract protein features. With these features, our model was trained to predict each parameter (IC_{50} , EC_{50} , and K_d) simultaneously as multitask learning (30). It allowed prediction of the IC_{50} , EC_{50} , and K_d values for one drug-protein pair.

Connectivity map analysis. The Connectivity Map (CMap) CLUE web application (<https://clue.io>) was used to assess the connectivity of gene expression signatures between chemical and genetic perturbations (31). The Touchstone app of the CLUE database provided connectivity scores ranging from -100 to +100, where positive scores corresponded to the positive correlation of gene signatures between the two perturbagens. Signatures with connectivity scores >40 (similar) were further investigated in this study.

Cell culture and reagents. NCI-H1299 (H1299; human non-small cell lung cancer cells), MDA-MB-231 (MB231; human breast adenocarcinoma cells), SCC4 (human tongue squamous cell carcinoma cells), and CAL27 (human tongue squamous cell carcinoma cells) cells were obtained from the Korean Cell Line Bank (Seoul, Korea) and American Type Culture Collection (Manassas, VA, USA). H1299 and MB231 cells were maintained in RPMI 1640 (HyClone, Logan, UT, USA), and SCC4 cells were maintained in DMEM: F12 medium (Gibco, Waltham, MA, USA) containing 10% fetal bovine serum (Gibco) and 1% antibiotics at 37°C in the presence of 5% CO₂. Fostamatinib (#S2625), BMS-777607 (#S1561), TAK-659 (#S8442), and MG-132 (#M7449) were purchased from Selleckchem (Houston, TX, USA).

Cell viability assay. Cell viability was evaluated using a CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega, San Luis Obispo, CA, USA). Cells were seeded at 3×10^3 cells/well in 96-well plates and then exposed to fostamatinib, BMS-777607, or TAK-659 for 48 h. Assays were performed by adding CellTiter 96® AQueous One Solution Reagent directly to the wells, incubating them for 2 h, and then measuring absorbance at 490 nm with a 96-well plate reader.

Western blotting and densitometric analysis. Cells were lysed in RIPA buffer (Elpis Biotech, Daejeon, Korea; #EBA-1149). Cell lysates were separated using SDS-PAGE, transferred onto PVDF membranes, and then incubated with primary antibodies followed by HRP-conjugated second antibodies (Genetex, Irvine, CA, USA). Chemiluminescent signals were visualized using the NEW Clarity ECL substrate (GE Healthcare, Chicago, IL, USA). Antibodies for phospho-AXL (#5724), AXL (#8661), and TYRO3 (#5585) were purchased from Cell Signaling Technology (Beverly, MA, USA). Antibodies for phospho-MERTK (#ab14921), MERTK (#ab52968), phospho-Syk (#ab62338), Syk (#ab3993), and α -tubulin (#ab4074) were purchased from Abcam (Cambridge, MA, USA). Western blot quantification was performed using ImageJ software (version 1.51, National Institutes of Health, Bethesda, MD, USA). The intensity of each protein band was quantified by measuring the integrated density value using ImageJ software. Background intensity was subtracted from each band intensity. The relative protein expression was calculated by normalizing the target protein intensity to that of

Table I. List of drugs with connectivity scores >40 after knock-down of TAM kinases.

Drug	DTI scores (predicted IC ₅₀ , nM)			Connectivity scores			MoA	FDA-labeled indication
	AXL	MERTK	TYRO3	AXL	MERTK	TYRO3		
Afatinib	619.3	700.7	601.5	68.6	-	94.0	EGFR inhibitor	Untreated, metastatic non-small cell lung cancer
Neratinib	156.3	349.1	158.8	67.6	45.6	-	EGFR inhibitor	HER2-overexpressed/amplified breast cancer
Axitinib	127.5	98.0	184.9	60.0	68.7	45.7	VEGFR inhibitor	Advanced renal cell carcinoma
Ruxolitinib	740.3	114.9	498.8	43.6	73.6	60.8	JAK inhibitor	Intermediate or high-risk myelofibrosis, including primary myelofibrosis
Bosutinib	123.6	126.4	73.9	40.7	51.9	51.9	Src/Abl inhibitor	Chronic myelogenous leukemia
Ceforanide	358.0	553.9	516.9	-	51.9	-	Antibacterial	Antibacterial
Zafirlukast	643.8	623.7	748.4	-	47.9	-	Leukotriene D4 receptor antagonist	Asthma
Selumetinib	191.3	336.3	186.9	-	-	46.6	MEK inhibitor	Neurofibromatosis type 1
Fostamatinib	754.4	867.8	978.4	-	-	40.5	Syk inhibitor	Immune thrombocytopenia

DTI, drug-target interaction; MoA, mechanism of action; FDA, Food and Drug Administration; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; VEGFR, vascular endothelial growth factor receptor; Syk, spleen tyrosine kinase.

α -tubulin from the same sample. Data from three independent experiments were analyzed and presented as mean $\pm$ standard deviation (SD).

Statistical analysis. All experiments were performed independently at least three times. Results are presented as the mean $\pm$ SD. Statistical significance was calculated by unpaired Student's t test using GraphPad Prism (La Jolla, CA, USA).

Results

MT-DTI predicts commercially available TAM kinase triple inhibitor candidates. To find an FDA-approved drug that can simultaneously target triple TAM kinases, we performed *in silico* screening using a deep learning-based model (23). By comparing the values predicted through MT-DTI (predicted IC₅₀ and predicted EC₅₀), drugs with an inhibitory effect twice that of the activating effect were selected. Thirty-one drugs with an IC₅₀ of less than 1 μ M in AXL, MERTK, and TYRO3 remained out of 2,459 FDA drug sets (Table SI). Of these 31 compounds, 21 were kinase inhibitors, including multi-kinase inhibitors (e.g. ponatinib, cabozantinib, lenvatinib), epidermal growth factor receptor (EGFR) inhibitors (e.g. neratinib, osimertinib, afatinib), and MEK inhibitors (e.g. selumetinib, trametinib, binimetinib, cobimetinib). In addition to kinases, 10 drugs included androgen receptor antagonists (e.g. apalutamide, enzalutamide), an antihypertensive (e.g. sitaxentan), and antibiotics (e.g. ceforanide, ozenoxacin). To explore the effects of each drug on TAM signaling, we analyzed the CMap resource. The CMap database, developed by the Broad Institute, is a collection of genome-wide gene expression data encompassing 1.5 million gene expression profiles from ~5,000

drug perturbagens and ~3,000 genetic perturbagens (32). CLUE (<https://clue.io/>) is a cloud-based software platform that provides a connectivity score between a query and a perturbagen and allows users to access publicly available CMap data (33). We compared the gene signatures induced by 31 drug treatments with the gene signatures by knock-down of AXL, MERTK, and TYRO3 using the Touchstone app in the CLUE platform, which shows connectivity between perturbagens. Gene signature scores for nine drugs among 31 compounds showed >40 connectivity with gene knock-down signature scores (Table I). This suggests that the nine drugs predicted by MT-DTI may act as potential inhibitors of TAM kinases. According to the FDA labeling information, afatinib, neratinib, axitinib, and bosutinib have already been approved for the treatment of various types of tumors, suggesting there is a limit to the concept of expansion of the drug indication through repurposing. In addition, ruxolitinib resulted in an additive effect by combined treatment with the AXL inhibitors, TP-0903 and bemcentinib (34,35). Because ceforanide and zafirlukast do not have a kinase inhibitor structure, the possibility of direct action on the TAM family is relatively low. By contrast, because fostamatinib, a Syk inhibitor, is already used as a treatment for ITP, and there are no reports of the effect of fostamatinib on the TAM family, we sought to verify the effect of fostamatinib on TAM signaling. The workflow of the current study is illustrated in Fig. 1.

Fostamatinib suppresses cell proliferation via inhibition of TAM kinases. The TAM family (AXL, MERTK, and TYRO3) are known to play an important role in the development of various cancers. Based on the results using MT-DTI, fostamatinib was predicted to be an inhibitor

of the TAM family. To determine the effect of the TAM family on solid cancers, experiments were conducted using the following cancer cell lines: H1299 cells (lung cancer); SCC4 cells (head and neck squamous cell carcinoma); and MB231 cells (breast cancer). We first evaluated the impact of BMS-777607, a known pan-TAM inhibitor, on cell viability and TAM protein expression in H1299, SCC4, and MB231 cells. BMS-777607 treatment resulted in a dose-dependent decrease in cell viability across all three cell lines (Fig. 2A). Western blot analysis revealed that BMS-777607 treatment led to reduced phosphorylation of both AXL and MERTK, indicating successful inhibition of these kinases (Fig. 2B). While we observed a decrease in total TYRO3 protein levels, we were unable to directly assess TYRO3 phosphorylation status due to technical limitations of available antibodies. This limitation stems from the high sequence homology between TYRO3 phosphorylation sites (Y681, Y685) and MERTK phosphorylation sites (Y749, Y753), which causes commercially available phospho-specific antibodies to cross-react with phosphorylated MERTK. Despite this technical constraint, the reduction in cell viability and clear effects on AXL and MERTK signaling demonstrate the effectiveness of BMS-777607 as a TAM family inhibitor. Based on these results, the same experiment was conducted using the cell lines listed above to confirm the role of fostamatinib in solid cancers (Fig. 2C and D). Cell viability was decreased on addition of increasing concentrations of fostamatinib, although there was a difference in degree. Both the phosphorylated and total forms of AXL and MERTK were decreased following fostamatinib treatment. These results suggest that fostamatinib reduces cell viability through the inhibition of TAM family proteins.

Other Syk inhibitors do not affect TAM signaling. Fostamatinib was previously known as a Syk inhibitor; therefore, we investigated whether other Syk inhibitors had the same effect on TAM signaling. TAK-659 is a potent and selective inhibitor of Syk. H1299, SCC4, and MB231 cells were assessed for cell viability and TAM family protein levels after TAK-659 treatment. H1299 and SCC4 cells did not show a change in cell viability on exposure to TAK-659, but MB231 cell viability was decreased slightly in a dose-dependent manner (Fig. 3A). Unlike the results after fostamatinib treatment, TAM family protein expression levels did not change after TAK-659 treatment in any of the cell lines tested (Fig. 3B). Basal levels of Syk protein expression were undetectable in H1299, SCC4, and MB231 cells, so we confirmed the effect of TAK-659 on TAM signaling using CAL27 cells, a cell line with high Syk expression. TAK-659 significantly reduced the Syk protein levels in CAL27 cells, whereas it did not affect the activity of AXL, MERTK, and TYRO3 (Fig. S1). These results suggest that the effect of fostamatinib on TAM signaling is a unique feature not found in other Syk inhibitors.

Discussion

Syk is a non-RTK and is known to play a crucial role in autoimmune diseases and hematological malignancies (1).

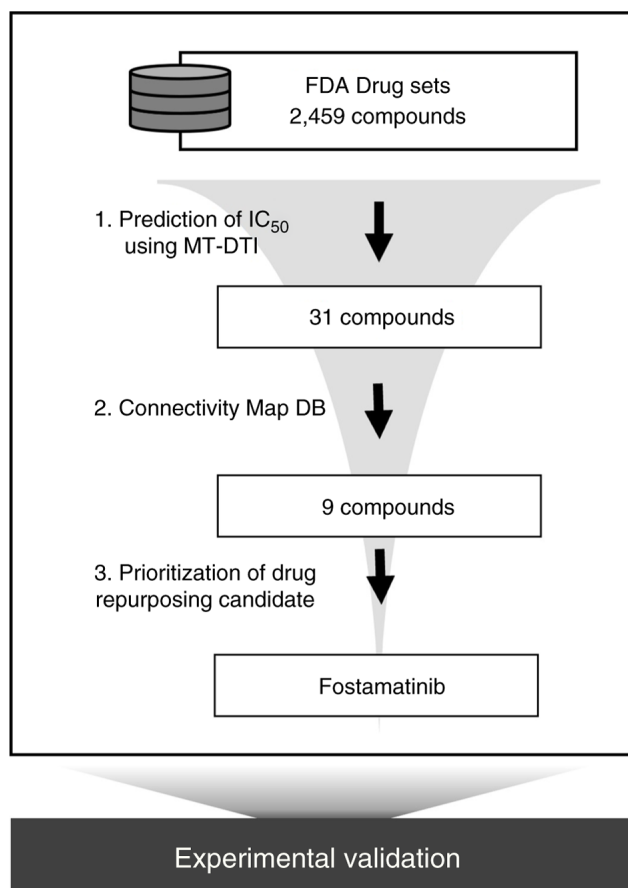


Figure 1. Workflow of the current study. The workflow mainly consists of a computational approach and experimental validation. A total of 31 Food and Drug Administration-approved drugs with an $IC_{50} < 1 \mu M$ in AXL, MERTK and TYRO3 were selected using the Deargen DTI model (MT-DTI). Next, transcriptome pattern analysis using the Connective Map database was performed. Fostamatinib, which was selected as the final candidate for drug repositioning, was experimentally validated. MT-DTI, molecule transformer-drug-target interaction; DB, database.

Syk mediates diverse biological functions, including innate immune recognition, platelet activation, cellular adhesion, osteoclast maturation, and vascular development (1,2). Fostamatinib is a prodrug that is converted to the active metabolite R406, which is a specific inhibitor of Syk-dependent Fcγ receptors-mediated signaling in a wide variety of tissues (36). In phase III randomized double-blind placebo-controlled FIT1 and FIT2 trials, the efficacy and safety of fostamatinib were demonstrated in patients with chronic ITP (37). Most adverse events were mild to moderate, and manageable. Based on these results, in 2018, the FDA approved fostamatinib for the treatment of chronic ITP in adults who had insufficient response to prior therapy (36,37). Although the clinical activity of fostamatinib has been reported in some hematological and solid cancers (8,9), the mechanism of action of fostamatinib, other than as a Syk kinase inhibitor, has not yet been described, and it has not been approved as an anticancer treatment. In this study, we discovered a novel function of fostamatinib by employing a drug repositioning approach via a machine learning algorithm.

An AI-driven drug repurposing approach, such as MT-DTI, and computational pharmacogenomics using large-scale

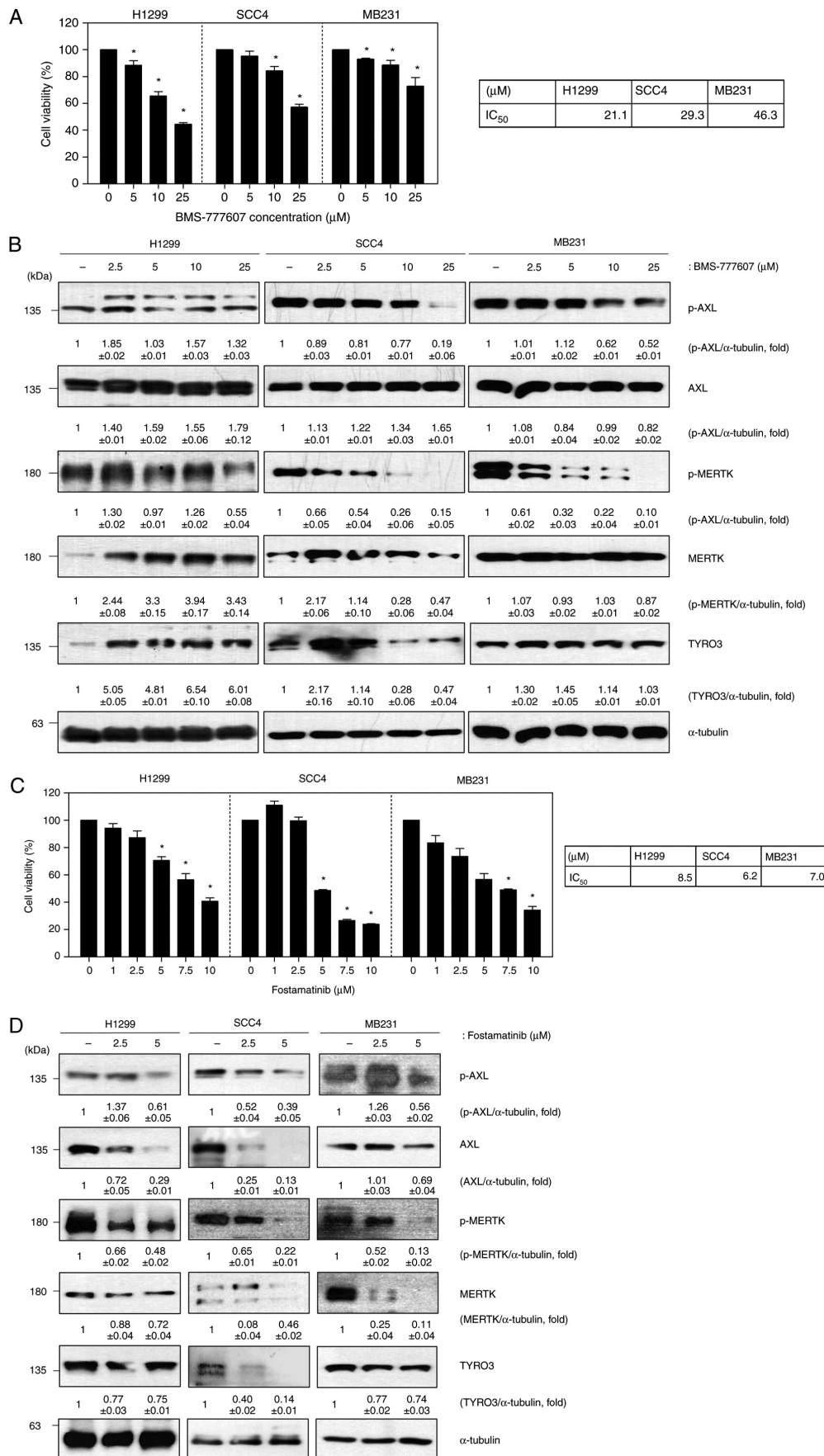


Figure 2. Fostamatinib suppresses cell proliferation via inhibition of TAM kinases. (A) Cell viability assay of H1299, SCC4 and MB231 cells after BMS-777607 treatment for 48 h; (B) Western blot of p-AXL, AXL, p-MERTK, MERTK and TYRO3 in BMS-777607-treated cells (α -tubulin was used as a loading control); (C) Cell viability assay of H1299, SCC4 and MB231 cells after fostamatinib treatment for 48 h; (D) Western blot of p-AXL, AXL, p-MERTK, MERTK and TYRO3 in fostamatinib-treated cells (α -tubulin was used as a loading control). Data are representative of three independent experiments and are presented as the mean $\pm$ SD. The statistical significance was analyzed via Student's t-test; * P <0.05 vs. untreated group. TAM, TYRO3, AXL, MERTK; p-, phosphorylated.

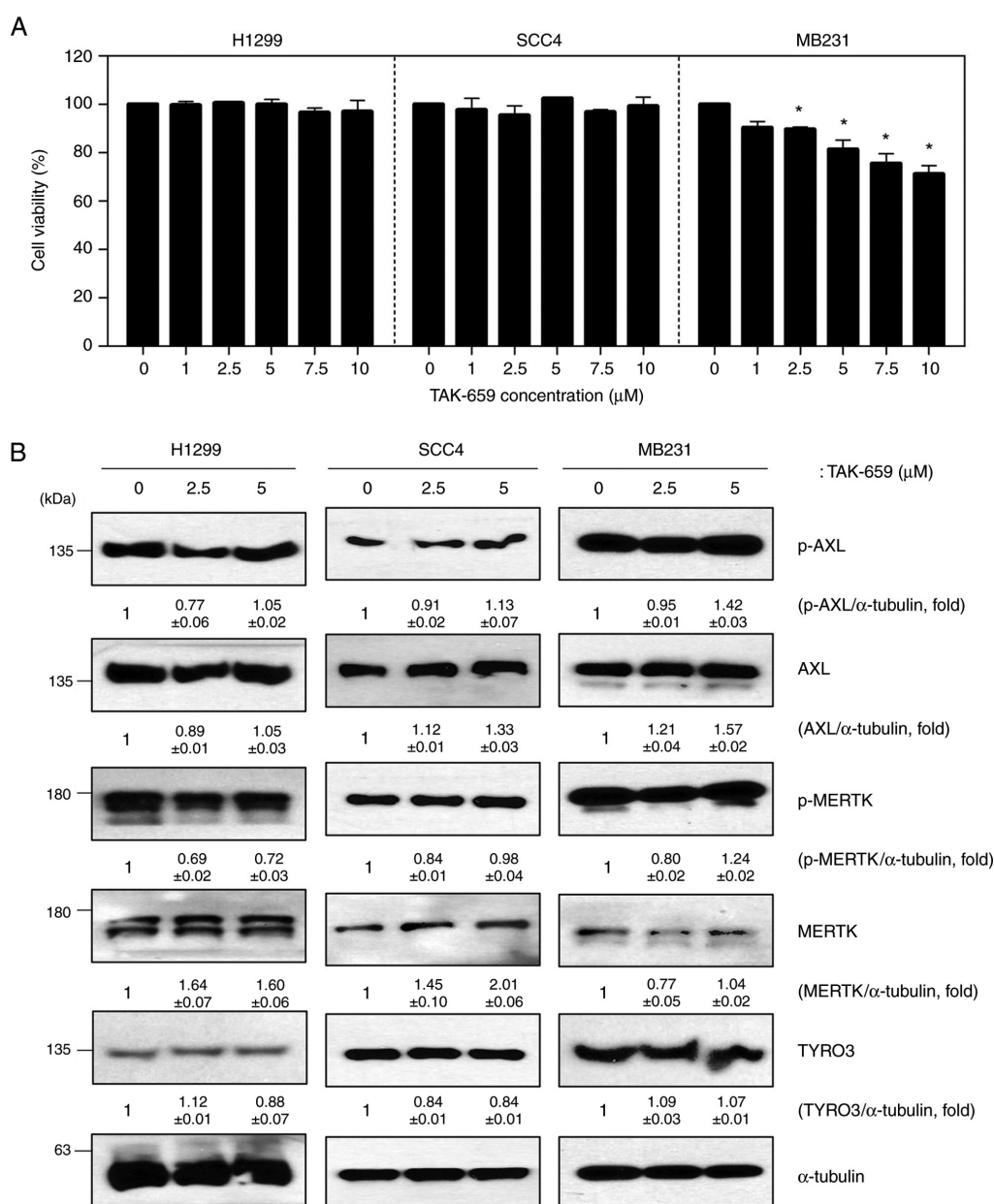


Figure 3. TAK-659, a Syk inhibitor, does not affect TAM signaling. (A) Cell viability assay of H1299, SCC4 and MB231 cells after TAK-659 treatment for 48 h; (B) Western blot of p-AXL, AXL, p-MERTK, MERTK, and TYRO3 in TAK-659-treated cells (α -tubulin was used as a loading control). Data are representative of three independent experiments and presented as the mean $\pm$ SD. The statistical significance was analyzed via Student's t-test. * $P < 0.05$ vs. untreated group. TAM, TYRO3, AXL, MERTK; p-, phosphorylated; Syk, spleen tyrosine kinase.

perturbation databases, provide effective drug discovery and drug repurposing/repositioning methodologies. As a result, we predicted fostamatinib to be a drug that can inhibit TAM RTKs. The inhibitory effect of fostamatinib on TAM kinases was verified with three different types of cancer cell lines: lung cancer (H1299), head and neck cancer (SCC4), and breast cancer (MB231). Fostamatinib significantly decreased the expression levels of AXL, MERTK, and TYRO3 equally in all three different types of cancer cell lines, and reduced cell viability in a dose-dependent manner. For each cell line, the IC_{50} values of fostamatinib were between 5 and 10 μ M, and the cytotoxic effect of fostamatinib was superior to that of the pan-TAM kinase inhibitor, BMS-777607. Regan-Fendt *et al.* (38) investigated the possibility of dasatinib and fostamatinib as a treatment for hepatocellular carcinoma

using a transcriptomics-based drug repurposing method. They showed that fostamatinib was able to inhibit the growth of hepatocellular carcinoma cells with IC_{50} values between 20 and 35 μ M (38). In the experiment using another well-known Syk inhibitor, the inhibitory effect on TAM kinases was not shown, indicating it is a unique characteristic of fostamatinib. Regarding the mechanism of action by which fostamatinib inhibits TAM receptors, we investigated the potential involvement of proteasomal degradation pathway. Our preliminary experiment with the proteasome inhibitor, MG132, showed that MG132 pretreatment could attenuate the fostamatinib-induced decrease in both phosphorylated and total protein levels of AXL and MERTK (data not shown). While these initial findings suggested that fostamatinib might promote TAM receptor degradation through a proteasome-dependent mechanism, we

faced significant technical challenges in fully validating this hypothesis. Specifically, when we attempted to optimize the MG132 and fostamatinib co-treatment conditions using H1299 cells, the combination showed considerable cellular toxicity that prevented us from obtaining reliable protein expression data, particularly for TYRO3 blots. This technical limitation, combined with the complex nature of receptor tyrosine kinase regulation, suggests that additional studies using alternative approaches would be needed to fully elucidate the precise mechanism by which fostamatinib regulates TAM receptor stability and function. Further investigation into the role of fostamatinib as a potential molecular glue degrader (MGD) might provide additional insights into its mechanism of action, as MGDs destabilize target proteins by bridging them near E3 ubiquitin ligases leading to polyubiquitylation and proteasomal degradation of the target protein.

The TAM family of RTKs, including AXL, MERTK, and TYRO3, is expressed in hematopoietic-derived cells that play important roles in efferocytosis, and in the balancing of immune responses and inflammation (10). TAM kinases are also overexpressed in a variety of cancers that are associated with chemoresistance, metastasis, and poor survival outcomes (10,11). As well as the promotion of tumor cell survival, proliferation, and invasion through activation of downstream oncogenic signaling pathways, TAM receptors contribute to tumor progression through an immunosuppressive tumor microenvironment and tumor immune escape (12-14). The key roles of TAM kinases as both oncogenic drivers and immune system regulators have led to a growing interest in TAM receptor inhibition, especially in the era of cancer immunotherapy. Of note, the TAM receptors, in particular MERTK, have been shown to upregulate the immune checkpoint molecule programmed cell death ligand 1 (PD-L1) in tumor cells (39). These data suggest that TAM tyrosine kinase inhibitors, in combination with immune checkpoint inhibitors (ICIs), could enhance treatment efficacy. In support of this hypothesis, Kasikara *et al* (40) reported that combined administration of an ICI with the pan-TAM tyrosine kinase inhibitor BMS-777607 enhanced tumor-infiltrating lymphocytes and T-cell-mediated immunity, with improved antitumor activity in a murine model of triple-negative breast cancer.

Several multitargeted tyrosine kinase inhibitors show the inhibitory effects of TAM kinases, but few have been specifically developed as TAM tyrosine kinase inhibitors. Several TAM-selective inhibitors are currently in development, and promising agents undergoing clinical trials include bemcentinib and BMS-777607 (10,13). Bemcentinib (also known as BGB324 or R428) is an orally available, selective inhibitor of AXL RTK that showed antitumor effects in preclinical models with multiple cancer cell lines (1,3,10). Bemcentinib also demonstrated synergistic effects when combined with ICIs, targeted therapies, and chemotherapeutic agents. As a result, this drug is now in phase I/II clinical trials for a variety of cancers, either alone or in combination with other chemotherapy regimens including ICIs (7,10,39). BMS-777607 was initially designed as a selective and orally available MET kinase inhibitor, but it was found to have a role as a potent pan-TAM inhibitor. BMS-777607 also demonstrated antitumor activity in *in vivo* models of several cancers, and it is now undergoing phase I/II clinical

trials in patients with advanced tumors (7,8,35,40). Despite some of these promising drugs, there are still unmet needs for more potent TAM kinase inhibitors with good safety profiles as new anticancer agents. The first consideration is the relative potency of the drug against the target. Previous studies indicate that inhibiting only one of three TAM receptors may not be effective because of the induction of compensatory activation of the other subunits (11,16). In preclinical models, MERTK inhibition alone was not sufficient to impact tumor growth, and some small-molecule inhibitors of AXL or MERTK showed low inhibitory potency against targets (7). In addition, some studies showed that AXL expression was increased after treatment with bemcentinib (41). This increase is caused by the inhibition of ubiquitination and degradation of AXL receptors by AXL phosphorylation blocking with small-molecule AXL inhibitors (42). Further research is needed to determine whether the paradoxical upregulation of AXL protein could have the potential to reverse any desired effect of treatment. Based on these previous data, we chose BMS-777607, the same pan-TAM inhibitor, instead of bemcentinib, a selective AXL inhibitor, to compare the efficacy of fostamatinib. Second, the potential concern of TAM kinase inhibition is about safety issues. TAM receptors are expressed in a wide range of tissues and have crucial roles in the maintenance of an organ's normal functions. Furthermore, because of their role in diminishing the innate immune response, any sustained TAM kinase inhibition may lead to adverse immune or inflammatory reactions, such as autoimmunity; thus, careful monitoring is required (39).

Taken together, TAM family kinases are distinct oncogenic drivers of novel anticancer targets through their promotion of tumor cell survival, proliferation, invasion, and chemoresistance, as well as their suppression of antitumor immunity. This study suggests that fostamatinib has the potential to be a novel anticancer agent through its inhibition of TAM tyrosine kinase. In addition, its efficacy is expected to be better than other previous TAM kinase inhibitors. An AI-driven drug-target interaction prediction model, such as MT-DTI, can contribute to the development of targeted therapy and be a powerful tool for drug repurposing. Further validation is warranted in animal models and clinical trials of patients with various types of cancer. Additional investigation into the role of fostamatinib in immune modulation in the tumor microenvironment, and in combination therapy with ICI, may provide clinically useful information for a new therapeutic strategy.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

YC, HSW and YHK conceptualized the study. The machine learning was performed by BL. BRB, HL, JHL, SK and SHC performed experiments and analyzed data. YC, HL, HSW and YHK wrote the original draft. YC, HSW and YHK reviewed and edited the manuscript. HSW and YHK acquired funding. YC and HSW confirm the authenticity of all the raw data. All authors have read approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

YC, BRB and BL were employed by the company Deargen Inc., who developed the deep DTI prediction model called Molecule Transformer (MT)-DTI employed in this study. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. All other authors do not have any financial and non-financial conflict of interest and declare that they have no competing interests.

References

- Graham DK, DeRyckere D, Davies KD and Earp HS: The TAM family: Phosphatidylserine sensing receptor tyrosine kinases gone awry in cancer. *Nat Rev Cancer* 14: 769-785, 2014.
- Wu G, Ma Z, Cheng Y, Hu W, Deng C, Jiang S, Li T, Chen F and Yang Y: Targeting Gas6/TAM in cancer cells and tumor microenvironment. *Mol Cancer* 17: 20, 2018.
- Lemke G and Rothlin CV: Immunobiology of the TAM receptors. *Nat Rev Immunol* 8: 327-336, 2008.
- Cook RS, Jacobsen KM, Wofford AM, DeRyckere D, Stanford J, Prieto AL, Redente E, Sandahl M, Hunter DM, Strunk KE, *et al*: MerTK inhibition in tumor leukocytes decreases tumor growth and metastasis. *J Clin Invest* 123: 3231-3242, 2013.
- Paolino M, Choidas A, Wallner S, Pranjic B, Uribealzo I, Loeser S, Jamieson AM, Langdon WY, Ikeda F, Fededa JP, *et al*: The E3 ligase Cbl-b and TAM receptors regulate cancer metastasis via natural killer cells. *Nature* 507: 508-512, 2014.
- Werfel TA and Cook RS: Efferocytosis in the tumor microenvironment. *Semin Immunopathol* 40: 545-554, 2018.
- Axelrod H and Pienta KJ: Axl as a mediator of cellular growth and survival. *Oncotarget* 5: 8818-8852, 2014.
- Holland SJ, Pan A, Franci C, Hu Y, Chang B, Li W, Duan M, Torneros A, Yu J, Heckrodt TJ, *et al*: R428, a selective small molecule inhibitor of Axl kinase, blocks tumor spread and prolongs survival in models of metastatic breast cancer. *Cancer Res* 70: 1544-1554, 2010.
- Crittenden MR, Baird J, Friedman D, Savage T, Uhde L, Alice A, Cottam B, Young K, Newell P, Nguyen C, *et al*: Mertk on tumor macrophages is a therapeutic target to prevent tumor recurrence following radiation therapy. *Oncotarget* 7: 78653-78666, 2016.
- Ludwig KF, Du W, Sorrelle NB, Wnuk-Lipinska K, Topalovski M, Toombs JE, Cruz VH, Yabuuchi S, Rajeshkumar NV, Maitra A, *et al*: Small-molecule inhibition of Axl targets tumor immune suppression and enhances chemotherapy in pancreatic cancer. *Cancer Res* 78: 246-255, 2018.
- Kim JE, Kim Y, Li G, Kim ST, Kim K, Park SH, Park JO, Park YS, Lim HY, Lee H, *et al*: MerTK inhibition by RXDX-106 in MerTK activated gastric cancer cell lines. *Oncotarget* 8: 105727-105734, 2017.
- Cummings CT, Zhang W, Davies KD, Kirkpatrick GD, Zhang D, DeRyckere D, Wang X, Frye SV, Earp HS and Graham DK: Small molecule inhibition of MERTK is efficacious in non-small cell lung cancer models independent of driver oncogene status. *Mol Cancer Ther* 14: 2014-2022, 2015.
- Gay CM, Balaji K and Byers LA: Giving AXL the axe: Targeting AXL in human malignancy. *Br J Cancer* 116: 415-423, 2017.
- Zhang W, DeRyckere D, Hunter D, Liu J, Stashko MA, Minson KA, Cummings CT, Lee M, Glaros TG, Newton DL, *et al*: UNC2025, a potent and orally bioavailable MER/FLT3 dual inhibitor. *J Med Chem* 57: 7031-7041, 2014.
- DeRyckere D, Lee-Sherick AB, Huey MG, Hill AA, Tyner JW, Jacobsen KM, Page LS, Kirkpatrick GG, Eryildiz F, Montgomery SA, *et al*: UNC2025, a MERTK small-molecule inhibitor, is therapeutically effective alone and in combination with methotrexate in leukemia models. *Clin Cancer Res* 23: 1481-1492, 2017.
- McDaniel NK, Cummings CT, Iida M, Hülse J, Pearson HE, Vasileiadi E, Parker RE, Orbuch RA, Ondracek OJ, Welke NB, *et al*: MERTK mediates intrinsic and adaptive resistance to AXL-targeting agents. *Mol Cancer Ther* 17: 2297-2308, 2018.
- Rios-Doria J, Favata M, Lasky K, Feldman P, Lo Y, Yang G, Stevens C, Wen X, Sehra S, Katiyar K, *et al*: A potent and selective dual inhibitor of AXL and MERTK possesses both immunomodulatory and tumor-targeted activity. *Front Oncol* 10: 598477, 2020.
- Ekins S, Puhl AC, Zorn KM, Lane TR, Russo DP, Klein JJ, Hickey AJ and Clark AM: Exploiting machine learning for end-to-end drug discovery and development. *Nat Mater* 18: 435-441, 2019.
- Issa NT, Stathias V, Schurer S and Dakshanamurthy S: Machine and deep learning approaches for cancer drug repurposing. *Semin Cancer Biol* 68: 132-142, 2021.
- Gilson MK, Liu T, Baitaluk M, Nicola G, Hwang L and Chong J: BindingDB in 2015: A public database for medicinal chemistry, computational chemistry and systems pharmacology. *Nucleic Acids Res* 44 (D1): D1045-D1053, 2016.
- Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, Li Q, Shoemaker BA, Thiessen PA, Yu B, *et al*: PubChem 2019 update: Improved access to chemical data. *Nucleic Acids Res* 47 (D1): D1102-D1109, 2019.
- Gaulton A, Bellis LJ, Bento AP, Chambers J, Davies M, Hersey A, Light Y, McGlinchey S, Michalovich D, Al-Lazikani B and Overington JP: ChEMBL: A large-scale bioactivity database for drug discovery. *Nucleic Acids Res* 40 (Database Issue): D1100-D1107, 2012.
- Beck BR, Shin B, Choi Y, Park S and Kang K: Predicting commercially available antiviral drugs that may act on the novel coronavirus (SARS-CoV-2) through a drug-target interaction deep learning model. *Comput Struct Biotechnol J* 18: 784-790, 2020.
- Choi Y, Shin B, Kang K, Park S and Beck BR: Target-centered drug repurposing predictions of human angiotensin-converting enzyme 2 (ACE2) and transmembrane protease serine subtype 2 (TMPRSS2) interacting approved drugs for coronavirus disease 2019 (COVID-19) treatment through a drug-target interaction deep learning model. *Viruses* 12: 1325, 2020.
- Connell NT and Berliner N: Fostamatinib for the treatment of chronic immune thrombocytopenia. *Blood* 133: 2027-2030, 2019.
- Tanoli Z, Alam Z, Vähä-Koskela M, Ravikumar B, Malyutina A, Jaiswal A, Tang J, Wennerberg K and Aittokallio T: Drug target commons 2.0: A community platform for systematic analysis of drug-target interaction profiles. *Database (Oxford)* 2018: 1-13, 2018.
- Liu T, Lin Y, Wen X, Jorissen RN and Gilson MK: BindingDB: A web-accessible database of experimentally determined protein-ligand binding affinities. *Nucleic Acids Res* 35 (Database Issue): D198-D201, 2007.
- Zhou J, Cui G, Hu S, Zhang Z, Yang C, Liu Z, Wang L, Li C and Sun M: Graph neural networks: A review of methods and applications. *AI Open* 1: 57-81, 2020.

29. Elnaggar A, Heinzinger M, Dallago C, Rihawi G, Wang Y, Jones L, Gibbs T, Feher T, Angerer C, Steinegger M, *et al*: ProtTrans: Towards cracking the language of life's code through self-supervised deep learning and high performance computing. arXiv preprint arXiv: 200706225, 2020.
30. Ruder S: An overview of multi-task learning in deep neural networks. arXiv preprint arXiv: 170605098, 2017.
31. Duan Q, Flynn C, Niepel M, Hafner M, Muhlich JL, Fernandez NF, Rouillard AD, Tan CM, Chen EY, Golub TR, *et al*: LINCS canvas browser: Interactive web app to query, browse and interrogate LINCS L1000 gene expression signatures. Nucleic Acids Res 42 (Web Server Issue): W449-W460, 2014.
32. Lamb J, Crawford ED, Peck D, Modell JW, Blat IC, Wrobel MJ, Lerner J, Brunet JP, Subramanian A, Ross KN, *et al*: The connectivity map: Using gene-expression signatures to connect small molecules, genes, and disease. Science 313: 1929-1935, 2006.
33. Subramanian A, Narayan R, Corsello SM, Peck DD, Natoli TE, Lu X, Gould J, Davis JF, Tubelli AA, Asiedu JK, *et al*: A next generation connectivity map: L1000 platform and the first 1,000,000 profiles. Cell 171: 1437-1452.e17, 2017.
34. Beitzten-Heineke A, Berenbrok N, Waizenegger J, Paesler S, Gensch V, Udonta F, Vargas Delgado ME, Engelmann J, Hoffmann F, Schafhausen P, *et al*: AXL inhibition represents a novel therapeutic approach in BCR-ABL negative myeloproliferative neoplasms. Hemasphere 5: e630, 2021.
35. Yan D, Earp HS, DeRyckere D and Graham DK: Targeting MERTK and AXL in EGFR mutant non-small cell lung cancer. Cancers (Basel) 13: 5639, 2021.
36. Markham A: Fostamatinib: First global approval. Drugs 78: 959-963, 2018.
37. Bussel J, Arnold DM, Grossbard E, Mayer J, Trelinski J, Homenda W, Hellmann A, Windyga J, Sivcheva L, Khalafallah AA, *et al*: Fostamatinib for the treatment of adult persistent and chronic immune thrombocytopenia: Results of two phase 3, randomized, placebo-controlled trials. Am J Hematol 93: 921-930, 2018.
38. Regan-Fendt K, Li D, Reyes R, Yu L, Wani NA, Hu P, Jacob ST, Ghoshal K, Payne PRO and Motiwala T: Transcriptomics-based drug repurposing approach identifies novel drugs against sorafenib-resistant hepatocellular carcinoma. Cancers (Basel) 12: 2730, 2020.
39. Aehnlich P, Powell RM, Peeters MJW, Rahbech A and Thor Straten P: TAM receptor inhibition-implications for cancer and the immune system. Cancers (Basel) 13: 1195, 2021.
40. Kasikara C, Davra V, Calianese D, Geng K, Spires TE, Quigley M, Wichroski M, Sriram G, Suarez-Lopez L, Yaffe MB, *et al*: Pan-TAM tyrosine kinase inhibitor BMS-777607 enhances anti-PD-1 mAb efficacy in a murine model of triple-negative breast cancer. Cancer Res 79: 2669-2683, 2019.
41. Chen F, Song Q and Yu Q: Axl inhibitor R428 induces apoptosis of cancer cells by blocking lysosomal acidification and recycling independent of Axl inhibition. Am J Cancer Res 8: 1466-1482, 2018.
42. Lauter M, Weber A and Torka R: Targeting of the AXL receptor tyrosine kinase by small molecule inhibitor leads to AXL cell surface accumulation by impairing the ubiquitin-dependent receptor degradation. Cell Commun Signal 17: 59, 2019.



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