1 Computational drug repositioning of bortezomib to reverse metastatic effect of
2 GALNT14 in lung cancer

3 Running title: A data-driven route to drug discovery for undruggable targets

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1 **Abstract**

2 Although many molecular targets for cancer therapy have been discovered, they often
3 show poor druggability, which is a major obstacle to develop targeted drugs. As an
4 alternative route to drug discovery, we adopted an *in silico* drug repositioning (*in silico*
5 DR) approach based on large-scale gene expression signatures, with the goal of
6 identifying inhibitors of lung cancer metastasis. Our analysis of clinicogenomic data
7 identified GALNT14, an enzyme involved in O-linked N-acetyl galactosamine
8 glycosylation, as a putative driver of lung cancer metastasis leading to poor survival. To
9 overcome the poor druggability of GALNT14, we leveraged Connectivity Map
10 approach, an *in silico* screening for drugs that are likely to revert the metastatic
11 expression patterns. It leads to identification of bortezomib (BTZ) as a potent metastatic
12 inhibitor, bypassing direct inhibition of poorly druggable target, GALNT14. The anti-
13 metastatic effect of BTZ was verified *in vitro* and *in vivo*. Notably, both BTZ treatment
14 and *GALNT14* knockdown attenuated TGF β -mediated gene expression and suppressed
15 TGF β -dependent metastatic genes, suggesting that BTZ acts by modulating TGF β
16 signaling. Taken together, these results demonstrate that our *in silico* DR approach is a
17 viable strategy to identify a candidate drug for undruggable targets, and to uncover its
18 underlying mechanisms.

19
20 **Keywords:** GALNT14, connectivity map, drug repositioning, bortezomib, TGF β ,
21 metastasis, undruggable targets

22

1 Introduction

2 In the context of personalized anti-cancer therapy based on targeting specific
3 proteins with the goal of lowering cancer-related mortality (1), a great deal of effort has
4 been devoted to identifying both molecular targets and accompanying drugs (2, 3).
5 However, the fraction of patients eligible for personalized anti-cancer therapy is very
6 limited (4) due to the poor druggability of newly identified molecular targets,
7 notwithstanding recent advances in strategies in drugging ‘undruggable’ proteins (5, 6).

8 An alternative approach to matching candidate drugs to poorly druggable
9 cancer targets is *in silico* drug repositioning (*in silico* DR) (7, 8). Due to the well-
10 characterized pharmacology and safety of approved drug libraries(9), this approach has
11 the potential to reduce cost and attrition during the clinical phases of drug development.
12 Several approaches to DR have been tested in the context of oncology (10) and a few of
13 the resultant drugs, including celecoxib and thalidomide, have been approved by the
14 FDA for repurposing as anti-cancer therapeutics (11). Along with recent advances in
15 sequencing technologies, chemogenomic databases containing drug-induced gene
16 expression profiles provide clues regarding potential treatments for personalized cancer
17 targets, and can also suggest candidate drugs based on tailored gene signatures of
18 cancers upon identification of molecular targets (12). The Connectivity Map (CMap), a
19 collection of genome-wide expression profiles of cell lines treated with >20,000
20 chemicals(13), has been used to identify candidate drugs for certain cancer types (14-
21 16).

22 N-acetyl-galactosaminyltransferases (GalNAc-Ts or GALNTs) are key
23 enzymes that initiate O-linked N-acetyl galactosamine (GalNAc) glycosylation. This
24 process is an important step in the synthesis of Thomsen-nouvelle (Tn) antigens, which

are well-characterized tumor-associated molecules (17). In particular, *GALNT14* has been examined in the context of apoptotic signaling (18, 19); invasion and migration of breast (20, 21), ovary (22), and lung (23) cancers; and multi-drug resistance of breast cancer cells (24). Moreover, *GALNT14* expression is not only a prognostic marker in neuroblastoma (25) and lung cancer (23), but also a predictive marker for Apo2L/TRAIL-based cancer therapy (18), although a randomized phase II study based on the predictive marker of GALNT14 for dulanermin did not improve patient outcome (26).

In this study, through transcriptome analysis of the TCGA dataset and *in vitro* and *in vivo* studies, we demonstrated that *GALNT14* is strongly associated with lung cancer recurrence due to the high migration and invasion properties of tumor cells. Rather than attempting direct inhibition of the poorly druggable GALNT14 protein or downstream signaling, we leveraged large-scale drug-induced transcriptome data to identify candidate drug(s) likely to reverse *GALNT14*-dependent gene expression, *i.e.*, drugs that led to transcriptomic changes similar to those induced by *GALNT14* depletion. We successfully identified an anti-metastatic candidate drug that mimicked *GALNT14* depletion. The results demonstrate that this approach represents a viable strategy for discovering candidate drugs for many other undruggable targets.

1 **Results and discussion**

2 ***GALNT14* as a putative molecular target for lung cancer metastasis**

3 Identification of molecular targets in recurrent cancers is essential not only for
 4 predicting prognosis, but also for matching specific drug–target pairs if they are
 5 available. To identify potential molecular targets related to cancer recurrence, we
 6 assembled transcriptome data and clinical information from 516 lung cancer patients
 7 from the TCGA LUAD cohort (Figure. 1A). Concentrating on molecular targets
 8 relevant to recurrent lung cancer, we performed a series of relapse-free survival (RFS)
 9 analyses and differential expression analyses. Expression of seven genes (*GALNT14*,
 10 *COL7A1*, *GPR115*, *C1QTNF6*, *KRT16*, *INHA*, and *TNFSF11*) was significantly
 11 associated with cancer progression, recurrence (Figure. 1B), and overall survival
 12 (Figure. S1A), indicating that these genes are potentially valuable as predictors of poor
 13 prognosis. Notably, metastasis-related genes were significantly overrepresented in gene
 14 lists selected by both RFS ($P = 2.3 \times 10^{-6}$) and differential expression ($P = 5.2 \times 10^{-19}$)
 15 analysis, including two genes (*e.g.*, *TNFSF11* and *INHA*) among the seven
 16 aforementioned candidates. To further confirm the relevance of each gene to metastasis
 17 or tumorigenesis, we divided lung cancer patients into two groups (low or high) using
 18 the median expression of each gene as the cutoff. Both metastasis and tumor signatures
 19 were positively enriched in the high-expression groups of all seven genes (Figure. 1C),
 20 and the significant enrichment was observed for *GALNT14* (Figure. 1D).

21 *GALNT14*, which encodes a glycosyltransferase involved in O-glycosylation,
 22 has been implicated in both tumor malignancy (25) and metastasis (20, 21, 23). As
 23 expected, metastatic (Figure. 1E) and tumorigenic potentials (Figure. 1F) were
 24 markedly attenuated by loss of *GALNT14*, indicating that the gene is important for

1 metastasis as well as tumorigenesis. Further, metastatic lung cancer cells were more
 2 vulnerable to *GALNT14* depletion than non-metastatic or other types of cancers in the
 3 *Project Achilles* dataset(27), a genome-scale RNAi screening data from for 501 cancer
 4 cell lines, including 126 cell lines originating from metastatic patients (Figure. 1G).
 5 Other candidate genes were less vulnerable in metastatic lung cancer (Figure. S1B).
 6 Consistent with this, *GALNT14* expression shows a clearly negative correlation with
 7 both locoregional recurrence-free survival (LRFS) and distant metastasis-free survival
 8 (DMFS) (Figure. 1H) as well as overall survival (Figure. S1A) in the TCGA LUAD
 9 cohorts. In addition, normal lung expresses only a low level of *GALNT14*, and there is a
 10 large gap between normal and lung cancer tissue (Figure. S1C)(28). All these results
 11 suggested *GALNT14* as a promising molecular target for lung cancer metastasis to
 12 improve patient survival.

13

14 **Computational repositioning of BTZ to reverse the *GALNT14* expression signature**

15 Although *GALNT14* may be a potential therapeutic target for metastatic lung cancer,
 16 GALNTs remain poorly druggable despite several attempts to find specific inhibitors
 17 (29, 30). Notably in this regard, GALNT14-dependent metastatic potential is governed
 18 by induction of transcription factors (e.g. *HOXB9* or *SOX4*) rather than by altered
 19 glycosylation (20, 23). Therefore, rather than inhibiting GALNT14 directly, we
 20 leveraged CMap dataset to virtually screen drugs that mimic the effects of *GALNT14*
 21 depletion at the transcriptome level. In particular, we focused on genes associated with
 22 metastasis or *GALNT14* expression, and considered that these genes should be
 23 suppressed in order to restore the metastatic to the normal. To this end, we defined two
 24 distinct *GALNT14* signatures. We collected a comprehensive list of 3711 metastasis-

1 related genes and selected two sets of genes among them: (i) 20 genes up-regulated in
2 the *GALNT14*-high group in the TCGA LUAD cohort, and (ii) 49 genes down-regulated
3 by *GALNT14* knockdown in the H460 cell line. Accordingly, our subsequent predictions
4 using *GALNT14* signatures would prioritize drugs that are probably relevant to both
5 *GALNT14*-dependence and metastasis.

6 Using the two *GALNT14* signatures, we performed two independent predictions by
7 CMap analyses (Figure. 2A). Candidate drugs were prioritized according to their DR
8 scores (See Materials and Methods in detail). Two drugs, dexamethasone (DEX, an anti-
9 inflammatory corticosteroid) and bortezomib (BTZ, a first-in-class proteasome inhibitor
10 used to treat multiple myeloma), were among the top candidates in both predictions
11 (Figure. 2B). We then validated expression levels of *SOX4*, *AREG*, and *VCAN*, which
12 are strongly associated with metastasis and regulated by *GALNT14* (20, 23) (Figures.
13 S2B and S2C). Among genes differentially expressed in response to either DEX or BTZ
14 in the CMap dataset, *SOX4* (but not *AREG* or *VCAN*) was commonly altered, indicating
15 that *SOX4* could serve as a validation marker (Figure. S2C). As predicted, we observed
16 dose-dependent suppression of *SOX4* (but not *VCAN*) in H460 cells treated with either
17 DEX or BTZ. Although BTZ suppressed *SOX4* less effectively than DEX (EC₅₀: 15 nM
18 vs. 5 nM, respectively) (Figure. S2D), BTZ treatment led to a significant reduction in
19 the migration capacity of H460 cells, whereas DEX did not (Figure. 2C). The EC₅₀ for
20 proteasome inhibition was around 20 nM (Figure. 2D and S2E), a concentration at
21 which BTZ clearly inhibited migration (Figures. 2E and S2F) and invasion (Figure. 2F)
22 by lung cancer cells, while it did not affect *GALNT14* expression (Figure. S2G), cell
23 viability (Figures. 2G and S2H) nor the cell proliferation (Figures. 2H and S2I). Of note,
24 A549 with relatively lower *GALNT14* expression than H1299 and H460 (Figure. S2J)

1 did not respond to BTZ treatment in migration (Figure. S2K) and invasion (Figure.
2 S2L), while proteasome inhibition by BTZ occurred (Figure. S2M), suggesting that
3 anti-invasion/migration effect of BTZ would be associated with GALNT14 expression.

5 **The effect of BTZ is independent of proteasome inhibition**

6 Given that the anti-migration/invasion effect of BTZ occurred at a
7 concentration that also inhibited proteasome activity (Figures. 2D and E), we sought to
8 determine whether this effect was a result of proteasome inhibition *per se*. To
9 investigate this issue, we first compared the chemical structure of three FDA-approved
10 proteasome inhibitors, BTZ, carfilzomib (CFZ), and ixazomib (IXZ), all of which are
11 approved for treatment of multiple myeloma (31). CFZ displayed similar profiles with
12 regards to Tanimoto coefficients or Jaccard index of molecular fingerprints(32)
13 (Figure. 3A). In contrast to BTZ, CFZ also inhibited proteasome activity (Figure. S3A)
14 and stabilized well-characterized proteasome targets β -catenin, Cyclin D1, and p27
15 (Figure. 3B), but failed to suppress migration (Figure. 3C) and invasion (Figure. 3D) at
16 the same concentration. Moreover, the boronic acid moiety responsible for the
17 proteasome inhibition (33) was present in both BTZ and IXZ. However, like CFZ,
18 treatment with IXZ could not inhibit migration (Figure. S3B). These data suggest that
19 BTZ has an off-target effect that is independent of proteasome inhibition.

20 To confirm that the anti-migration/invasion effect of BTZ is not dependent on
21 proteasome inhibition, we compared transcriptome profiles of H460 cells treated with
22 BTZ, CFZ and depleted of *GALNT14* (sh*GAL*). Perturbation by BTZ and sh*GAL* (but
23 not CFZ) induced similar transcriptomic changes relative to the control (Figure. 3E, left
24 panel). Moreover, the expression patterns of metastatic signature genes were even more

similar between BTZ and shGAL, whereas CFZ had a minimal effect on only a few genes (Figure. 3E, middle panel). By contrast, expression of proteasome-related genes was altered significantly by both drugs, but only marginally by shGAL (Figure. 3E, right panel). Genes down-regulated by BTZ and shGAL overlapped significantly (101 common genes; $P = 5.3 \times 10^{-54}$), suggesting that BTZ treatment partially mimics depletion of *GALNT14* (Figure. 3F). These results are consistent with the phenotypic outcomes, i.e., BTZ, but not CFZ, suppressed cell migration and invasion similarly to *GALNT14* depletion.

Attenuation of the TGF β gene response by BTZ treatment or *GALNT14* knockdown

We hypothesized that a subset of the 101 genes down-regulated by both BTZ treatment and *GALNT14* depletion could account for anti-migration/invasion effects of BTZ. To investigate the drug's mode of action, we conducted pathway enrichment analysis of the 101 genes and investigated the clinical significance of each pathway (Figure. 4A). Among the most enriched pathways was TGF β signaling (hazard ratio [HR] = 1.2). BTZ treatment induced changes in expression of individual TGF β signaling genes that were very similar to those induced by shGAL (Figure. S4A for BTZ and Figure. S4B for shGAL). Moreover, genes commonly down-regulated (e.g., *INHBA*, *FST*, and *BMPR*) among targets of TGF β signaling were indeed suppressed (Figure. 4B). Suppression of the TGF β -dependent gene signature, a common effect of BTZ treatment and *GALNT14* depletion, was validated by reporter assays using the Smad-binding element (SBE), activin-response element (ARE), and BMP-response element

1 (BRE) (Figure. 4C). Similarly, TGF β reporter activity decreased after treatment with
2 BTZ, but not CFZ (Figure. 4D), while β -catenin and Cyclin D1 were stabilized by
3 proteasome inhibition following treatment with either BTZ or CFZ (Figure. S4C).
4 Receptors activation by TGF β transduce signal through direct phosphorylation of R-
5 SMAD, including SMAD2 or SMAD3, which leads to interaction to co-SMAD (i.e.
6 SMAD4) for nuclear translocation (34). Concomitant with the reduction in SMAD2
7 phosphorylation with (Figure. 4E) or without TGF β stimulation (Figure. S4C), SMAD4
8 nuclear translocation was inhibited significantly by BTZ treatment (Figure. 4F).
9 Consistently, TGF β dependent gene responses (determined by SBE or BRE) upon
10 TGF β stimulation, were significantly attenuated by BTZ treatment (Figure. S4D). As
11 depletion of SMAD4 was sufficient to inhibit both migration and invasion (Figures. 4G
12 and H), inhibition of SMAD2 phosphorylation (Figures. 4E and S4C) and subsequent
13 delay of SMAD4 nuclear translocation (Figure. 4F) by BTZ would be a possible mode
14 of action of BTZ. It is important to note that the TGF β signaling pathway has been
15 studied extensively as a tumor suppressor, a tumor promoter (35), and a promoter of
16 metastasis (36). To determine whether the TGF β signaling response is associated with
17 *GALNT14*, we selected a set of TGF β downstream targets (37) and examined their
18 correlations with *GALNT14* expression and patient prognosis (Figure. 4I). Notably,
19 SMAD4-dependent targets *PCDH7* and *LAMC2*, previously shown to induce metastasis
20 (38, 39) or tumorigenicity (40, 41), were highly correlated with *GALNT14* (Figure. 4I).
21 Moreover, the SMAD4-dependent TGF β targets was associated to RFS in the
22 *GALNT14*-high group ($P = 0.045$, Figure. 4J), suggesting that some SMAD4-dependent
23 targets responsible for cancer recurrence are strongly associated with *GALNT14*

1 expression. These results imply that BTZ treatment, like *GALNT14* depletion, exerts its
 2 anti-metastatic effect by interfering with nuclear translocation of SMAD4 (Figure. 4F)
 3 and with the SMAD4-dependent gene expression response (Figure. 4D). Finally, we
 4 defined a set of genes commonly down-regulated by both BTZ and sh*GAL* among the
 5 SMAD4 dependent targets as ‘*GALNT14*–TGFβ signature’ (See Materials and Methods).
 6 The average activity of the *GALNT14*–TGFβ signature strongly discriminated patient
 7 RFS ($P = 4.0 \times 10^{-4}$, Figure. 4K), and *GALNT14* expression was significantly higher in
 8 lung cancer patients with higher levels of the signature ($P = 0.025$, Figure. 4L). Overall,
 9 these results suggest that suppressing TGFβ signaling and gene expression responses
 10 relevant to BTZ (similar to the response observed after *GALNT14* depletion) makes a
 11 major contribution to reducing migration and invasion.

12

13 ***In vivo* validation of ant-metastatic effect of BTZ**

14 Given the anti-migration/invasion effect of BTZ in a lung cancer cell model (Figure. 2),
 15 we next tested the *in vivo* efficacy of BTZ against cancer metastasis *in vivo*. For this
 16 purpose, local metastasis was induced in mice by tail vein injection of H460 lung cancer
 17 cells, followed by treatment with or without BTZ or CFZ twice weekly for three weeks
 18 (Figure. 5A). The proteasome-inhibitory effect of BTZ (0.1mg/kg) with CFZ (0.5 mg/kg)
 19 was examined by measuring proteasome activity in blood (Figure. 5B). Under this
 20 concentration, the mice tolerated both BTZ and CFZ, exhibiting neither significant loss
 21 of body weight nor any other abnormalities (Figure. 5C). Consistent with the *in vitro*
 22 assay, the number of metastatic nodules in the lungs of BTZ-treated mice was
 23 significantly lower than that in CFZ- or vehicle-treated animals (Figure. 5D, S5A and
 24 S5B). Close examination of cancer tissue also revealed that inflammatory lesions, which

1 provide favorable microenvironments for tumor formation (42), were present in both
 2 CFZ- and vehicle-treated mice (Figure. S5C). These results were validated with further
 3 experiment to support the efficacy of BTZ on metastasis inhibition. BTZ treatment
 4 considerably attenuated lung colonization (Figure. 5E and S5D) in the presence of a
 5 proteasome inhibitory effect of BTZ (Figure. 5F) but no physiological abnormality
 6 (Figure. S5E). Taken together, the *in vivo* and *in vitro* data reveal that BTZ has a
 7 significant therapeutic advantage over CFZ in that it inhibits cancer metastasis without
 8 significant undesirable side effects.

9 Although many molecular targets for tumorigenesis and metastasis have been
 10 identified, most remain undruggable. For example, the GALNTs, expression of which is
 11 strongly associated with various properties of cancer (20-25), have yet to be drugged,
 12 although a few attempts have been made to develop inhibitors of GALNT-dependent O-
 13 linked glycosylation (29, 30). Thereby, instead of searching direct inhibitors of
 14 GALNT14, we adopted an *in silico* DR approach to reverse the *GALNT14*-dependent
 15 metastatic expression signature with the goal of finding a candidate drug that could
 16 interfere with the *GALNT14*-dependent cancer phenotype. Notably, *HOXB9* and *SOX4*,
 17 transcription factor genes regulated by *GALNT14*, are responsible for metastasis (23)
 18 and self-renewal (20), respectively, suggesting that downstream transcriptional
 19 modulation would be a promising strategy.

20 Unlike similar studies in the past that used the CMap method to analyze differences
 21 in gene expression signatures between normal and cancerous tissue (14, 43), we focused
 22 exclusively on genes (e.g., *GALNT14*) related to the pertinent phenotype (*i.e.*, the
 23 metastatic gene signature) (Figure. 1) and identified BTZ as a drug candidate with novel
 24 anti-metastatic effects both *in vitro* (Figure. 2C–2H) and *in vivo* (Figure. 5). Importantly,

1 we also demonstrated that the anti-metastatic effect of BTZ, in contrast to that of CFZ,
2 was independent of proteasome inhibition (Figure. 3). Moreover, the metastatic gene
3 signature of CFZ was also distinct from that of BTZ, whereas the proteasome gene
4 signatures of the two drugs were relatively similar (Figure. 3). Recent studies examined
5 the inhibitory effect of BTZ on TGF β -dependent responses such as fibrosis (44) and
6 survival of lymphoma (45), and the results support our conclusions. We also identified
7 the *GALNT14*-TGF β signature that serves as clear indicators of poor prognosis (Figure.
8 4). Thus, attenuation of TGF β signaling by BTZ, depletion of *GALNT14*, or inhibition
9 of TGF β signaling all decrease invasive properties *in vitro* (Figure. 4) and lung
10 metastasis *in vivo* (Figure. 5), suggesting the drug's mode of action. Accordingly, the
11 *GALNT14*-TGF β signature represents potentially useful prognostic marker for lung
12 cancer patients, and could be used alongside previously reported marker [*e.g.*, the
13 TGF β -response signature (TBRS) (46) and the MAPK pathway activity score (MPAS)
14 (47)]. Of note, although BTZ had no undesirable side effects in our *in vivo* experiments,
15 the risk of peripheral neuropathy in patients treated with BTZ (48) merits a further
16 search for other candidate drugs with safer profiles that could also reverse the gene
17 signature associated with *GALNT14* or *GALNT14*-TGF β activity. The continued search
18 for improved candidates could be performed using recently reported *in silico* (or
19 computational) DR tools (8, 49, 50). Notably in this regard, a recent *in silico* approach
20 can predict candidate drugs capable of modulating the activities of oncogenic
21 transcription factors, a class of proteins that has yet to be drugged (7). In the future, it
22 would be interesting to apply this type of approach to modulate *GALNT14*-regulated
23 transcription factor genes such as *HOXB9* and *SOX4*, which mediate metastasis (23) and

1 self-renewal (20), respectively. Besides predicting potential candidate drugs, our *in*
2 *silico* DR approach enabled identification of several marker genes that turned out to be
3 strongly associated with clinical outcomes such as RFS. This strategy, which integrated
4 multiple independent expression signatures from cancer patients, genetic perturbation
5 (*e.g.*, knockdown or overexpression), and drug treatment (CMap), would be applicable
6 generally to any other types of target. Thus, our results provide a strong “proof-of-
7 concept” that our DR method is a viable strategy for accessing undruggable molecular
8 targets, leading to identification of candidate drugs that target specific cellular processes
9 such as cancer metastasis.

10

11

1 **Methods**

2 **Cell line establishment and Cell culture**

3 H460, A549 and H1299 cell line which was purchased from Korean cell line bank
 4 (KCLB) were maintained in Dulbecco's modified Eagle's medium (DMEM),
 5 supplemented with 10% fetal bovine serum (FBS), gentamicin (50 µg/ml) at 37°C in a
 6 humidified atmosphere of 5% CO₂ in the air. *GALNT14* knockdown cell lines with
 7 shRNA were established as previously described (23).

8 **Reagents and antibodies**

9 The primary antibodies against cleavage caspase-3 (#9664), cleavage caspase-9 (#9505)
 10 and pSmad2 (#310S) were obtained from Cell Signaling Technology. Antibodies against
 11 β-Actin (sc-47778), PARP (sc-7150), p53 (sc-126), p27 (sc-528), p21 (sc-397),
 12 CyclinD1 (sc-718) and CyclinB1 (sc-245) were obtained from Santa Cruz
 13 Biotechnology Inc. and β-catenin (BD 610153) was purchased from BD Biosciences
 14 pharmigen. Bortezomib (S1013) and Carfilzomib (S2853) were purchased from
 15 selleckchem.

16 **RNA-sequencing and analysis**

17 H460 cells treated with BTZ, CFZ, depleted of *GALNT14* (sh*GAL*) and their control
 18 (DMSO, shCont) were prepared for RNA sequencing. Total RNA was isolated using the
 19 Trizol according to the manufacture instruction. For library construction, we used the
 20 TruSeq Stranded mRNA Library Prep Kit (Illumina, San Diego, CA). Briefly, the steps
 21 of strand-specific protocol are: first strand cDNA synthesis; second strand synthesized
 22 using dUTPs instead of dTTPs; end repair, A-tailing, and adaptor ligation; PCR
 23 amplification. Then, each library was diluted to 8 pM for 76 cycles of paired-read
 24 sequencing (2 X 75bp) on the Illumina NextSeq 500 per the manufacturer's

1 recommended protocol. Read sequences were aligned to the reference genome (UCSC
2 hg19) and the mapped counts per gene were quantified by STAR (51). The raw counts
3 were normalized to CPM (counts per million) based on the trimmed mean of M-values
4 (TMM) normalization method using R package 'edgeR'. Differential expression
5 analyses between samples (shCont vs shGAL, DMSO vs BTZ, or DMSO vs CFZ) were
6 performed through DESeq2 (52).

7 **TCGA data processing and analysis**

8 TCGA lung adenocarcinoma (LUAD) cohort (n=576), containing mRNA gene
9 expression and clinical data on 388 primary lung cancers, 128 lung cancers with
10 recurrence, and 59 benign lung tissues were collected from the Broad GDAC Firehose
11 (<https://gdac.broadinstitute.org/>). Total 494 patients with clinical information tracked for
12 at least one month were used for survival analysis. For 20,531 genes, all patients were
13 divided into high and low groups by the median expression of each gene and relapse-
14 free survival analysis was performed according to the group difference. Hazard ratio
15 and *P* value were calculated by Cox proportional hazards regression model and log-rank
16 test respectively. With 58 patients who have gene expression profiles of normal benign
17 tissues, differential expression in LUAD compared to matched normal samples were
18 measured from the likelihood ratio test. RNA-seq profiles were normalized and
19 processed using R package 'limma' and 'DESeq2', and survival analysis was conducted
20 by R package 'survival'.

21 **Metastasis and Tumorigenesis signatures**

22 From MSigDB manually curated gene sets (C2), we collected the 35 metastasis- and
23 44 tumor-related gene sets that are annotated by 'metastasis' / 'epithelial-mesenchymal
24 transition' and 'cancer' / 'tumorigenesis', respectively. We took only Up-regulated genes

1 when both UP- or DOWN-regulated sets were available. Then, we selected 32 and 23
2 genes as the metastatic and the tumorigenesis signature, respectively, by taking the
3 consensus genes common to at least three or more gene sets.

4 **CMap analysis to predict candidate drugs**

5 The updated CMap dataset, or LINCS L1000, provides an extensive catalog of
6 transcriptome profiles for 71 human cell lines treated by 20,413 small molecules. We
7 obtained the expression dataset (level 5, replicate-collapsed z-score) from Gene
8 Expression Omnibus (GSE92742), each of which represents normalized genome-wide
9 differential expression profiles under a unique experimental condition (cell line, drug,
10 treated dose/time, batch). We used only the 60,321 profiles of high-quality
11 ($\text{distil_cc_q75} \geq 0.2$, $\text{pct_self_rank_q25} \leq 0.05$, and $\text{distil_nsample} \geq 3$) among the
12 total 205,034 profiles. Unlike the original CMap based on microarray, a single drug may
13 have multiple expression profiles depending on cell line, dose, time, and sample batch.
14 Therefore, instead of using the original rank-based Kolmogorov-Smirnov (KS) test, we
15 made a modified analytic scheme to handle such redundancy. For prediction of drugs
16 mimicking the down-regulation of *GALNT14*-associated metastatic genes (i.e.
17 *GALNT14* signature), we calculated Jaccard index between the *GALNT14* signature and
18 the 100 most down-regulated genes of CMap profiles as similarity score (S). Then, the
19 similarity scores of a drug were merged into a single DR score (drug repositioning
20 score), which was calculated as the negative logarithm of the hypergeometric P-value
21 for over-representation of its similarity scores within the top 10% of the total similarity
22 scores.

23 **GALNT14-TGF β signature**

TGF β downstream target genes (39 SMAD4 dependent genes and 65 SMAD4 independent genes) were collected from the literature (37). Among the SMAD4 dependent targets, a set of genes commonly down-regulated by both BTZ and shGAL (*ATF3*, *ARNTL*, *COPA*, *NEDD9*, *LAMC2*, *RAB27B*, and *PCDH7*) was defined as ‘GALNT14–TGF β signature’. To measure the average activity of the signature, KS statistic was used to estimate the degree of up- or down-regulation of the seven genes in a sample’s gene expression profile. In TCGA LUAD cohort, the patients with activity in the top 10% (n=50) or lower 10% (n=50) were classified as ‘high’ or ‘low’ respectively.

Statistical analysis

The graphical data were presented as mean $\pm$ S.E.M. Statistical significance among the three groups and between groups was determined using one-way or two-way analysis of variance (ANOVA) following Turkey post-test and Student’s t-test respectively. Significance was assumed for $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***).

SUPPLEMENTARY DATA

Supplementary information is available from the Wiley Online Library or from the author.

AUTHORS’ CONTRIBUTIONS

HJ.C and W.K conceived the overall study design and led the experiments. OS.K and H.L mainly conducted the experiments, data analysis, and critical discussion of the results. HJ.K, JE.P, and W.L conducted the mouse xenograft experiments. S.K, JH.K and M.K generated and analyzed RNAseq data. All authors contributed to manuscript

1 writing and revising, and endorsed the final manuscript.

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5

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9

10 **DISCLOSURE DECLARATION**

11 The authors declare that they have no conflict of interest.

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5

1 **Figure Legends**

2 **Figure 1 *GALNT14* as a putative molecular target for lung cancer metastasis**

3 **A.** Identification of therapeutic targets for both cancer progression and recurrence based
4 on transcriptomic analysis of lung cancer patients. **B.** Selection of therapeutic target
5 candidates that are differential expressed between tumor and normal tissue as well as
6 significantly associated to RFS in the 516 patient dataset from TCGA LUAD. Red
7 circles indicate metastasis-related genes annotated by MSigDB. **C.** Enrichment analysis
8 of metastatic and tumor signatures between high- and low-expressed patient groups for
9 each of the seven candidate genes. **D.** The normalized enrichment score (NES) was
10 calculated by Gene Set Enrichment Analysis (GSEA) for the metastasis (left) and
11 tumorigenesis signature (right) after all the genes were ranked by their expression fold
12 change. **E-F.** Control H460 (shCont) and *GALNT14* knockdown (shGal) cells were
13 injected into lateral tail vein (E) or flanks (F) of nude mice. The representative H&E
14 staining images of tumor-bearing lung (E) and tumors (F) were presented. **G.**
15 Comparison of *GALNT14* dependencies among metastatic and primary cell lines from
16 lung and other types of cancer from the *Project Achilles* dataset. Only metastatic lung
17 cells show a significant dependency on *GALNT14*. **H.** Comparison between the high-
18 and low- expression group for *GALNT14* in terms of locoregional recurrence-free
19 survival (LRFS), and distant metastasis-free survival (DMFS) in LUAD patients.

20 **Figure 2 Computational repositioning of BTZ to reverse the *GALNT14* expression** 21 **signature**

22 **A.** CMap analyses to prioritize anti-metastatic candidate drugs using the two
23 independent *GALNT14* signatures. Candidate drugs were prioritized according to the
24 similarities between drug-induced down-DEGs and the two *GALNT14* signatures. **B.**

1 DR score of top candidate drugs selected by the *GALNT14* signatures from TCGA (x-
2 axis) and H460 (y-axis). The two drugs marked in red (dexamethasone and bortezomib)
3 got high scores in both predictions. **C.** Representative microscopic images of cell
4 migration after treatment of indicated dose of (nM) of BTZ and DEX (left), Relative
5 recovery ratio of indicated group was graphically presented. (right, n.s: not significant)
6 **D.** Proteasome activity in H460 cells after indicated dose of BTZ was graphically
7 presented. **E.** Representative microscopic images of cell migration at 43 hours after
8 treatment of indicated dose of (nM) of BTZ (left), Relative recovery ratio of indicated
9 dose was graphically presented (right) **F.** Representative image of invaded cell through
10 two-chamber model after DMSO or 20 nM of BTZ treatment (left), Relative invasion
11 area was graphically presented (right). **G.** Flow cytometry for Annexin V and 7-AAD at
12 24 hours after indicated dose of BTZ (top), Graphical presentation of live (white box)
13 and dead (black box) was shown (bottom). **H.** Flow cytometry of cell cycle profile at 24
14 hours after indicated dose of BTZ treatment (left), Graphical presentation of each cell
15 cycle (G1, S and G2/M) were shown (right).

16 **Figure 3 The effect of BTZ is independent of proteasome inhibition**

17 **A.** Chemical structure of proteasome inhibitors (bortezomib, carfilzomib, and ixazomib)
18 and their Tanimoto similarity heatmap. Red circle indicates the boronic acid structure. **B.**
19 Immunoblotting for β -catenin, Cyclin D1 and p27 after treatment of BTZ (20 nM) and
20 CFZ (20 nM), α -tubulin for equal protein loading control **C.** Representative
21 microscopic images of cell migration at 43 hours after 20 nM of BTZ and CFZ
22 treatment (left), Relative recovery ratio was graphically presented (right, n.s: not
23 significant). **D.** Representative image of invaded cell through two-chamber model
24 after DMSO or 20 nM of BTZ or CFZ treatment (left), Relative invasion area was

graphically presented (right, n.s.: not significant). **E.** Sample clustering using t-SNE based on the expression of whole genes, metastasis-related genes and proteasome-related genes. Each sample was colored according to its perturbation type. **F.** Venn diagram of differentially down-regulated genes by BTZ, CFZ and shGAL perturbation in the gene space of whole genes.

Figure 4 Attenuation of the TGF β gene response by BTZ treatment or *GALNT14* knockdown

A. Enriched pathways in the 101 genes common to the down-regulated signatures by BTZ treatment and *GALNT14* knockdown (hypergeometric test, P value < 0.01 and FDR < 0.1), and the distribution of RFS HR for the pathway genes in TCGA LUAD cohort (red circle: the median HR of the pathway genes, horizontal bar: the range of HR in each pathway). **B.** mRNA expression of TGF-beta signaling genes among the 101 common signature genes by *GALNT14* depletion and BTZ treatment. **C.** Relative luciferase reporter activity (SBE, ARE and BRE) of shRNA control (shCont: white box) and *GALNT14* knockdown (shGal: black box) H460 cells was graphically presented. **D.** BRE luciferase activity at 24 hours after 20 nM of BTZ or CFZ treatment (n.s.: not significant) **E.** Immunoblotting analysis for phosphorylated SMAD2 (pSMAD2) and phosphorylated ERK2 (pERK2) pretreated with DMSO or 20 nM of BTZ at indicated time after TGF β (10 ng/ml) treatment, β -actin used as an equal protein loading control **F.** Fluorescent microscopic images for SMAD4 (green) after 20nM of BTZ treatment, DAPI (blue) for nuclear counterstaining **G-H** Representative microscopic images of cell migration (G) and invaded cells of two-chamber invasion assay (H) at 48 hours after introduction of control siRNA (siN.C) or siRNA for SMAD4 (siSMAD4) (left), Relative recovery ratio (G) or invaded area (H) was graphically presented (right). **I-L.**

1 Analysis of TCGA LUAD cohort, **I.** The relationships between correlation score with
2 *GALNT14* expression (z-transform of Spearman correlation, x-axis) and RFS HR (y-
3 axis) of each gene in TGF β target genes. The dependency of TGF β target genes on
4 Smad4 was labeled with two different colors (yellow: Smad4-dependent, blue: Smad4-
5 independent). **J.** KM plot of RFS stratified by the combination of *GALNT14* expression
6 (low and high) and TGF β target activity (low and high). TGF β target activity was
7 measured using SMAD4 dependent genes (left) and independent genes (right)
8 individually. Significant differences between the patient groups were marked with the
9 asterisk (*). **K.** KM plot of RFS stratified by the *GALNT14*-TGF β signature. *P* values
10 and HR were calculated with the log-rank test and Cox regression, respectively. **L.**
11 Differences in *GALNT14* expression by *GALNT14*-TGF β signature in TCGA LUAD
12 cohort. *P* values were calculated with Student's t-test.

13 **Figure 5 *In vivo* validation of anti-metastatic effect of BTZ**

14 **A.** Schematic overview of *in vivo* experimental procedure **B-D.** Lateral tail vein
15 injection of vehicle (saline, n=9) or BTZ (0.1 mg/kg, n=7) or CFZ (0.5 mg/kg, n=7)
16 followed by H460 cells (B) Proteasome activity in whole blood collected 1 hour after
17 BTZ or CFZ was graphically presented. (P.C: positive control, described in material
18 method) (C) Body weight of each mouse monitored at indicated days, was graphically
19 presented (D) Representative images of whole lung of each group was presented (top).
20 White arrowheads indicate tumor nodule. Number of mice bearing tumor nodule was
21 shown in the table (bottom). **E-F** Lateral tail vein injection of vehicle (saline, n=7) or
22 BTZ (0.1 mg/kg, n=5) followed by H460 cells (E) Representative microscopic images
23 of lung tumor bearing lesion (left) and graphical presentation of the number of
24 metastatic tumor nodule (right) (F) Proteasome activity in whole blood collected 1 hour

- 1 after BTZ was graphically presented. (P.C: positive control, described in material
- 2 method)
- 3

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Figure. 1

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Figure. 4

K

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Figure. 5

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