Molecular Cancer Therapeutics

TTK Inhibitors as a Targeted Therapy for *CTNNB1* (β-catenin) Mutant Cancers

Guido J.R. Zaman, Jeroen A.D.M. de Roos, Marion A.A. Libouban, Martine B.W. Prinsen, Jos de Man, Rogier C. Buijsman, and Joost C.M. Uitdehaag

Abstract

The spindle assembly checkpoint kinase TTK (Mps1) is a key regulator of chromosome segregation and is the subject of novel targeted therapy approaches by small-molecule inhibitors. Although the first TTK inhibitors have entered phase I dose escalating studies in combination with taxane chemotherapy, a patient stratification strategy is still missing. With the aim to identify a genomic biomarker to predict the response of tumor cells to TTK inhibitor therapy, we profiled a set of preclinical and clinical TTK inhibitors from different chemical series on a panel of 66 genetically characterized cell lines derived from different tumors (Oncolines). Cell lines harboring activating mutations in the *CTNNB1* gene, encoding the Wnt pathway signaling regulator β-catenin, were on average up to five times more sensitive to TTK inhibitors than cell lines wild-type for *CTNNB1*. The association of *CTNNB1*-mutant status and increased cancer cell line sensitivity to TTK inhibition was confirmed with isogenic cell line pairs harboring either mutant or wild-type *CTNNB1*. Treatment of a xenograft model of a *CTNNB1*-mutant cell line with the TTK inhibitor NTRC 0066-0 resulted in complete inhibition of tumor growth. Mutations in *CTNNB1* occur at relatively high frequency in endometrial cancer and hepatocellular carcinoma, which are known to express high *TTK* levels. We propose mutant *CTNNB1* as a prognostic drug response biomarker, enabling the selection of patients most likely to respond to TTK inhibitor therapy in proof-of-concept clinical trials. *Mol Cancer Ther; 16(11); 2609–17. ©2017 AACR.*

Introduction

Targeted therapies exploit unique features of tumor cells, permitting cancer specificity, while having limited effects on healthy cells. Many targeted therapies are genotype-selective, enabling the selection of patients most likely to respond by the detection of specific genomic alterations in tumors, such as gene amplifications, translocations, or point mutations. Well-known examples are the use of growth factor receptor kinase inhibitors in breast cancer patients with amplification of the *HER2* gene (1), and inhibitors of a mutant form of the serine/threonine kinase B-RAF in metastatic melanoma (2).

The dual-specificity protein kinase TTK, commonly referred to as Mps1, is a component of the spindle assembly checkpoint, a surveillance mechanism that ensures the fidelity of chromosome segregation (3). Inhibition of TTK kinase activity with small-molecule kinase inhibitors leads to chromosome segregation errors by allowing mitotic exit in the presence of unattached kinetochores (4–6). After several rounds of cell division, the accumulation of chromosome segregation errors results in cancer cell death by apoptosis (7, 8). Several TTK inhibitors have been shown to reduce the growth of xenografts of human cancer cell lines from diverse tumor tissue origin in mice (5, 7–13). In an immunocompetent mouse model of triple-negative breast cancer (TNBC; ref. 8), and in patient-derived xenograft models (12), TTK inhibitors increased the efficacy of taxane chemotherapy.

Because of its role in proper chromosome segregation, TTK inhibition also kills nontransformed proliferating cells (8, 14), and concerns have been raised whether sufficient therapeutic window will be achievable in the clinic (11). However, we showed that mice could undergo repeated treatments with the selective small-molecule inhibitor NTRC 0066-0 over a period of more than 150 days in combination with docetaxel, resulting in tumor remission and without signs of toxicity (8). Four TTK inhibitors are currently evaluated in phase I dose escalating studies, some in combination with paclitaxel (refs. 12, 13; http://www.clinicaltrials.gov; clinical studies NCT02138812, NCT02366949, NCT02792465; EudraCT no.: 2014-002023-10).

Despite these advances, development of a patient stratification strategy would greatly help the applicability of TTK inhibitors in the clinic. However, unlike mutant BRAF in metastatic melanoma (2), there is no relationship between mutated or activated TTK and malignancy status known. Furthermore, whereas *TTK* is highly expressed in several cancer types, the relationship between expression level and severity of disease is complex and contradictive (14). For example, high *TTK* expression correlates with poor prognosis in hepatocellular carcinoma (HCC; ref. 15) and Her2-positive breast cancer (14), while low *TTK* expression correlates with poor patient outcome in triple-negative states (14). In the past, sensitivity to TTK inhibition has been associated with mutations in *TP53* or *PTEN* (16, 17), but these results have not been further confirmed (7, 13).

To enable the selection of patients most likely to respond to TTK inhibitor therapy, we profiled a set of ten preclinical and clinical inhibitors on a panel of 66 genetically characterized cancer cell lines derived from different tumor tissues (Oncolines; refs. 18,

Netherlands Translational Research Center B.V., Oss, the Netherlands.

Note: Supplementary data for this article are available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).

Corresponding Author: Guido J.R. Zaman, Netherlands Translational Research Center B.V., Kloosterstraat 9, 5349 AB Oss, the Netherlands. Phone: 314-1270-0500; Fax: 314-1270-0501; E-mail: guido.zaman@ntrc.nl

doi: 10.1158/1535-7163.MCT-17-0342

19). Drug sensitivity was related to the mutation status of 114 cancer genes in an unbiased way. Mutant *CTNNB1*, coding for the Wnt signaling pathway regulator β-catenin, was identified as a unique prognostic drug response biomarker for TTK inhibitors. Experiments with isogenic cell line pairs and a mouse xenograft model confirmed that mutant *CTNNB1* associates with sensitivity to TTK inhibitor therapy.

Materials and Methods

TTK inhibitors

All TTK inhibitors were synthesized in-house according to published procedures (5, 20–24), except Mps1-IN-1 (6) and TC-Mps1-12 (10), which were purchased from Tocris. Compounds were stored as solids at room temperature and dissolved in dimethyl sulfoxide before experiments.

Kinase assays

Binding affinity (K_d) for TTK was determined by surface plasmon resonance (8). Selectivity over Aurora A, Aurora C, and Polo-like kinase 1 (PLK1) was determined in enzyme assays performed at $K_{M,ATP}$ using full-length enzymes from Carna and Lance time-resolved fluorescence (PerkinElmer). The inhibitor constant (K_i) was calculated from the half-maximum inhibitory concentration (IC_{50}) with the Cheng–Prusoff equation.

Cancer cell lines

Cancer cell lines were obtained from the ATCC from 2011 to 2014 and cultured in ATCC-recommended media. All experiments were carried out within nine passages of the original vials from ATCC who authenticated all cell lines by short tandem repeat analysis. The mutation status of all cell lines was obtained from the Catalogue of Somatic Mutations in Cancer (COSMIC) version 75 (http://cancer.sanger.ac.uk/cosmic; ref. 25) and was filtered as outlined earlier (19). The mutant status of twenty-five well-known cancer driver genes, including *CTNNB1* (Table 1), was verified from samples generated at NTRC by DNA sequence analysis (19). Isogenic cell lines derived from HCT116 and lacking either the mutated *CTNNB1* gene copy (−/+) or the wild-type (ΔS45/-; ref. 26) were purchased from Horizon Discovery.

Differences in Wnt pathway activation were verified by immunoblot analysis of nuclear β-catenin and analysis of *Axin2* expression with quantitative PCR (qPCR). Primers used were: β-actin, forward: CAAGAGATGGCCACGGCTGCTTCCA; reverse, 5′-GCATGGAGTTGAAGGTAGTTTCG-3′; 18s, forward: 5′-AGACAACAAGCTCCGTGAAGA-3′; reverse, 5′-CAGAAGTGACGCAGCCCTCTA-3′; HPRT, forward: 5′-GACCAGTCAACAGGGGACAT-3′; reverse, 5′-CCTGACCAAGGAAAGCAAAG-3′; Axin2, forward: 5′-GGTGTTTGAGGAGATCTGGG3′; reverse, 5′-TGCTCACAGCCAA-GACAGTT3′.

Table 1. Mutations in *CTNNB1*-mutant cell lines used in this study

Cell line	Tumor origin	Mutation	Amino acid change
Cell lines in the Oncolines cancer cell line panel[a]			
A427	Lung carcinoma	121A>G	T41A
HCT116	Colon carcinoma	131–133ΔCTT	ΔS45
LS 174T	Colorectal adenocarcinoma	134C>T	S45F
SW48	Colorectal adenocarcinoma	98C>A	S33Y
Additional cell lines			
HuTu80	Duodenum adenocarcinoma	110C>T	S37F
TOV-112D	Ovary carcinoma	109T>G	S37A
TOV-21G	Ovary carcinoma	876A>T	K292N (non-hotspot)

[a]Mutation status was verified by DNA sequence analysis and found to be identical as reported in the COSMIC database version 75.

Cell proliferation assays

Cell proliferation assays were performed as described previously (18, 19). In brief, cells were seeded in a 384-well plate and incubated for 24 hours. After addition of compound solution, the plates were incubated for an additional 120 hours (5 days) after which 25 μL of ATPlite 1Step (PerkinElmer) solution was added. Luminescence was recorded on an Envision multimode reader (Perkin Elmer). Percentage growth compared with uninhibited control was used for curve fitting to determine IC_{50}s. For all analyses pIC_{50}s ($-^{10}\log IC_{50}$; in mol/L) were used, unless otherwise indicated.

Genomic biomarker analysis

Differences in pIC_{50} (ΔpIC_{50}) between "modified" and "wild-type" groups of cell lines were analyzed in three ways. First, cell lines were ranked on drug response in a waterfall plot. Second, a subset of the most commonly occurring cancer genes (23 in total; ref. 18) was analyzed by type II ANOVA analysis in the statistical program R (27). The result of the ANOVA analysis was visualized in a volcano plot. Third, a set of 114 cancer genes (19) was analyzed by a two-sided homoscedastic *t* test in R (27). The *P* values from ANOVA and *t* test were subjected to a Benjamini–Hochberg multiple testing correction, and only genetic associations with a false discovery rate less than 20% were considered significant. Differences between cell line subsets were further tested for significance with a one-sided Student *t* test in Microsoft Excel. In addition, we performed Wilcoxon rank sum tests in R (27). This test analyzes rankings and is less sensitive to outliers because it does not assume normal data distribution.

Gene set analysis

Gene expression profiles were downloaded for 57 of the 66 Oncolines cell lines, from the Cancer Cell Line Encyclopedia (CCLE; ref. 28). Subsequently, Pearson correlations were calculated between the expression levels of each individual gene and $^{10}\log(IC_{50})$s. The list was sorted from a high correlation between expression and sensitivity, to anticorrelation. This sorted list was used as input for Gene Set Analysis (GSA) with the package piano in R (27, 29). Gene sets were obtained from the MSigDB database (software.broadinstitute. org/gsea/msigdb). To limit the number of tested hypotheses, we restricted GSA to Wnt/β-catenin signaling-related sets from the Protein Interaction Database (PID) and Chemical and Genetical Perturbations (CGP) collections.

In vivo studies

Intervention studies in mouse xenograft models of the human β-catenin–mutant lung carcinoma cell line A-427 and the TNBC cell line MDA-MB-231 were performed at Charles River Laboratories. Experiments were conducted in accordance to protocols approved by the Charles River Discovery Services Animal Care and Use Committee. The animal care and use program at Charles River Discovery is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

A427 cells were implanted subcutaneously into nude mice on day 0. Once the tumors reached a volume of 150 mm^3, mice were treated with vehicle or 20 mg/kg NTRC 0066-0 administered orally every other day (Q2D, $n = 10$ mice per treatment group). Tumor growth was monitored using caliper measurement three times weekly. The study was terminated when tumor size of the vehicle-treated mice reached a maximum size of 750 mm^3. MDA-MB-231 cells were implanted orthotopically into nude mice on day 0. Once the tumors reached a volume of 200 mm^3, mice were treated with vehicle or 20 mg/kg NTRC 0066-0 orally every other day ($n = 10$ mice per treatment group). Tumor growth was monitored using caliper measurement three times weekly. The study was terminated when tumor size of the vehicle-treated mice reached a maximum size of 1,500 mm^3.

Statistical comparisons between compound-treated (NTRC 0066-0) and nontreated (vehicle) were analyzed by *t* test (unpaired, two-tailed) using GraphPad Prism StatView software (Abacus Concepts, Inc.). Relative inhibition of tumor growth (TGI) was calculated from the relative tumor volume (RTV) as follows: $TGI = 100 \times 1 - (TV_{f,treated} - TV_{i,treated})/(TV_{f,control} - TV_{i,control})$, where TV_f is the average tumor volume at the end of the study, and TV_i is the average tumor volume at the initiation of treatment (13).

Results

Activating mutations in *CTNNB1* correlate with cellular response to TTK inhibitors

We previously described the biological characterization of NTRC 0066-0, a subnanomolar potent inhibitor of TTK in enzyme activity and binding assays (8). The compound inhibited the proliferation of a wide variety of cancer cell lines with a potency similar to that of classic cytotoxic agents, and was efficacious in mouse cancer models, without toxicity (8). NTRC 0066-0 only kills proliferating cells, but there is no relationship between cell doubling time and half maximal inhibitory potency (IC_{50}) in cell proliferation assays (8, 30). Because cell line profiling is one of the best ways to discover if certain genomic alterations confer drug sensitivity (28), we extended the number of cancer cell lines examined from 45 to 66, and related sensitivity of the cell lines to the presence or absence of mutations in 23 known cancer genes (18). Cell lines were ranked on potency in a waterfall plot (Fig. 1A). Differences in inhibitory potency were related to cancer gene mutation status by ANOVA, and displayed in a volcano plot (Fig. 1B). This revealed that NTRC 0066-0 preferentially inhibits the proliferation of cell lines harboring a mutant copy of the *CTNNB1* gene, encoding the Wnt pathway regulator β-catenin (31). Mutant *CTNNB1* is represented by four cell lines in the panel (Table 1): the lung cancer cell line A427, the colon carcinoma cell line HCT116, and the colorectal adenocarcinoma cell lines LS 174T and SW48. These are indicated in red in the waterfall plot (Fig. 1A). The difference in sensitivity between *CTNNB1* wild-type and cell lines mutant for *CTNNB1*, expressed as a differences in $-^{10}\log IC_{50}$ (ΔpIC_{50}), was –0.49 and the difference in median values was –0.44 (Supplementary Tables S1A–S1C). This means that *CTNNB1*-mutant cell lines are on average three times more sensitive to NTRC 0066-0 than cell lines that are wild-type for this gene. All four cell lines contain point mutations or deletions in serine or threonine residues encoded by a cancer hotspot region in exon 3 of the *CTNNB1* gene (Table 1). Mutations in these regions impair the phosphorylation by upstream kinases that mark β-catenin for proteosomal degradation (32). Accumulated β-catenin transfers to the nucleus where it activates the transcription of Wnt-dependent genes, such as *MYC* (31).

To determine whether mutant *CTNNB1* is a common marker of increased tumor cell line sensitivity to TTK inhibitors, we further profiled a set of well-characterized TTK inhibitors based on different chemical scaffolds on the cell panel (Supplementary Fig. S1; Supplementary Table S1A). Compounds 4 and 5 are derivatives of NTRC 0066-0 with increased binding affinity for TTK (33). Mps-BAY2b (Bayer A.G.; refs. 7, 22) is a structural analogue of BAY 1217389 and based on a different chemical backbone than BAY 1161909 (refs. 12, 20–21; Supplementary Fig. S1). NMS-P715 (5) is a structural analogue of the clinical compound of Servier, S81694 (34). Tc-Mps1-12 (10), Mps1-IN-1 (6), and MPI-0479605 (9, 23) are research tool compounds.

The TTK selectivity of the different inhibitors has been demonstrated by profiling experiments on large kinase assay panels (5–10, 12). To compare them head-to-head, we further tested their activity in enzyme assays for Aurora A, Aurora C, and Polo-like kinase 1 (PLK1; Supplementary Table S1D). Although some inhibitors show cross-reactivity with Aurora A and PLK1, all compounds showed at least 100 times selectivity over Aurora A, Aurora C, and PLK1 (Supplementary Table S1D). Moreover, cellular IC_{50}s, especially for *CTNNB1*-mutant cell lines, are lower than the biochemical IC_{50}s for the off-targets, indicating that these play a negligible role in the antiproliferative effects of the inhibitors.

All TTK inhibitors preferentially inhibited *CTNNB1*-mutant cell lines in the ANOVA (Fig. 1C; Supplementary Fig. S2). Compound 5 showed the strongest targeting of 6 times (Supplementary Table S1B). The significance of the differences was confirmed in a Wilcoxon rank sum test, which is less sensitive to outliers (Supplementary Table S1C). Because the mutant cell lines are mostly from colon cancer origin, we compared the pIC_{50}s in mutant cell lines with wild-type colon cancer lines specifically. This confirmed the preferential sensitivity of *CTNNB1*-mutant lines to TTK inhibitors (Supplementary Tables S1E and S1F). To test if the effect was related to cell doubling time, we defined a subset of wild-type cell lines showing comparable or faster doubling rates than mutants (19), and used this set as comparator (Supplementary Table S1G). Also in this case, differences between mutant and wild-type lines remained significant. In fact, the cell line that was most sensitive to TTK inhibitors, A427, has a doubling time that is only average across the cell line panel (Supplementary Table S1H).

To determine the uniqueness of the association between TTK inhibitor sensitivity and mutant *CTNNB1*, we next related sensitivity to a broader set of, in total, 114 cancer genes that occur as variants in at least three of the 66 cell lines (ref. 19; Supplementary Table S1I). For NTRC 0066-0, this revealed one novel gene, in which mutation associated with sensitivity, that is, *ARHGAP35*, encoding Rho GTPase activating protein (ΔpIC_{50}, –0.48; $P_{adjusted} = 0.13$, mutated in four cell lines; Supplementary Table S1J). Mutation in this gene also related with sensitivity to other TTK inhibitors (Supplementary Table S1K). Notably, cell lines that are mutant for *APC*, encoding another regulator of the Wnt pathway, were also slightly more sensitive to NTRC 0066-0 (ΔpIC_{50}, –0.18), but the correlation was not statistically significant ($P_{adjusted} = 0.53$, 11 cell lines; Supplementary Table S1I). However, when

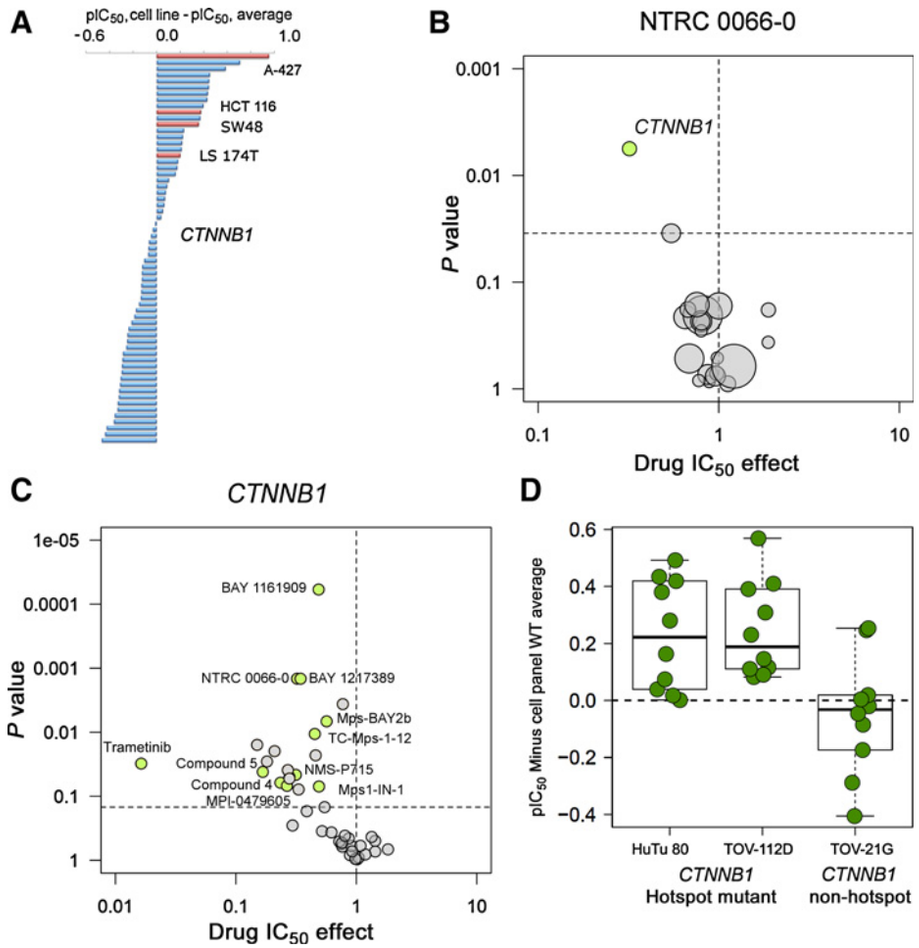

Figure 1.
Mutant *CTNNB1* correlates with tumor cell line sensitivity to TTK inhibitors. **A**, Waterfall plot ranking 66 cancer cell lines from the Oncolines panel on sensitivity (pIC_{50}) for the TTK inhibitor NTRC 0066-0 in cell proliferation assays. *CTNNB1*-mutant cell lines are indicated in red; wild-type in blue. The horizontal axis indicates the pIC_{50} in the indicated cell line minus the average pIC_{50} in the cell panel. **B**, Relationship between cancer cell line sensitivity for NTRC 0066-0 and mutant status of 23 frequently mutated cancer genes (18). The volcano plot shows the average IC_{50} shift between mutant and nonmutant cell lines (*x*-axis) and significance from the ANOVA test (*y*-axis). Significance was corrected for multiple testing and associations above the threshold (horizontal dotted line) are colored light green. Areas of circles are proportional to the number of cell lines carrying mutations. **C**, Gene-based volcano showing relative drug sensitivity of ten TTK inhibitors for *CTNNB1*-mutant cell lines in comparison with 122 anticancer agents profiled on the same panel (19). **D,** Validation of mutant *CTNNB1* as drug sensitivity biomarker for TTK inhibitors using additional cancer cell lines with mutations in exon 3 hotspot and non-hotspot regions. Every dark green dot represents one of the ten TTK reference inhibitors. pIC_{50}s were measured in triplicate and averaged. The difference is shown with the average pIC_{50} in the 62 *CTNNB1* wild-type cell lines in the Oncolines cell panel (represented by the horizontal dotted line).

CTNNB1 and *APC*-mutant cell lines are analyzed as one group, sensitivity differences are significant ($P_{adjusted} = 0.11$, 14 cell lines). The same trend was seen for the other TTK inhibitors (Supplementary Table S1K). Only NMS-P715, which has relatively low cellular potency, showed significant preferential targeting of *APC* alone, in addition to *CTNNB1* (Supplementary Fig. S2; Supplementary Table S1K).

It should be noted that, like others (7, 12, 13), we did not find a relationship between *TP53* and *PTEN* mutations and TTK inhibitor sensitivity, as reported in early studies (16, 17). Furthermore, in a genomic biomarker analysis of 122 anticancer agents (19), we only found MEK inhibitors that preferentially targeted *CTNNB1*, as previously reported (18), indicating that the observed *CTNNB1* targeting is not caused by the selection of cell lines in the panel, but a result of compound properties, namely TTK inhibition.

To investigate the influence of assay conditions and cell panel composition, we reanalyzed the profiling results reported by other groups working on TTK inhibitors (refs. 12, 34, 35; Supplementary Table S2A). All three panels include HCT116 and LS 174T, and two include SW48. Two panels included a cell line with a *CTNNB1* exon 3 hotspot mutant that was not included in the Oncolines cell panel. Reanalyzing the IC_{50}s in these studies with a Wilcoxon rank sum test revealed significant associations of cancer cell line sensitivity and mutant *CTNNB1* for the clinical compound BAY 1217389 (12) and for the preclinical compound CCT271850 (ref. 35; Supplementary Table S2B). Reanalyzing the data for NMS-P715 (34) and BAY 1161909 (12) moreover revealed a trend for *CTNNB1* lines to be more sensitive to these inhibitors.

To further scrutinize the relation between *CTNNB1* mutation and sensitivity to TTK inhibitors, we acquired two additional cell lines from the ATCC containing *CTNNB1* exon 3 hotspot mutations, and that were not part of the Oncolines panel. These are the duodenum carcinoma line Hutu 80 and the ovary carcinoma line TOV-112D (Table 1). As control, we also acquired TOV-21G, an ovary carcinoma line with a non-hotspot *CTNNB1* mutation. This was originally isolated in the same laboratory as TOV-112D, but from a different patient (Table 1). All TTK inhibitors were tested in proliferation assays in the three lines (Fig. 1D; Supplementary Table S3A). A Wilcoxon rank sum test across all inhibitors demonstrated that the *CTNNB1*-mutant cell lines HuTu 80 and TOV-112D were more sensitive than wild-type cell lines ($P = 0.01$ and $P = 0.005$, respectively), whereas the control cell line TOV-21G showed no difference in sensitivity ($P = 0.87$). This further validates mutations in the exon 3 hot spot region as a prognostic biomarker for TTK inhibitor sensitivity.

Validation of mutant *CTNNB1* as a marker of TTK inhibitor sensitivity using isogenic cell lines

To determine whether mutant *CTNNB1* was sufficient to confer increased sensitivity to TTK inhibitors, proliferation assays were performed with a pair of isogenic cell lines derived from the colon carcinoma cell line HCT116 (26). Parental HCT116 cells harbor a deletion of three base pairs in one copy of the *CTNNB1* gene, resulting in deletion of the regulatory serine residue at position 45 of β-catenin (Table 1). The genotype of the cell line for *CTNNB1* is referred to as ΔS45/+. Deletion of the serine impairs proteasome-mediated degradation, resulting in accumulation of β-catenin in the nucleus and increased Wnt pathway-regulated gene expression (26, 32) (Supplementary Fig. S3A).

Isogenic cells lacking the mutated *CTNNB1* gene copy (–/+) were less sensitive to TTK inhibitors than parental HCT116 cells (Fig. 2A), which are heterozygous for mutant *CTNNB1* (ΔS45/+). Difference in sensitivity, expressed as a shift in pIC_{50} (ΔpIC_{50}), was approximately one-half log unit for NTRC 0066-0 (ΔpIC_{50}, 0.43). In addition, the maximum effect at the highest compound concentration was decreased by 21% in the cell line lacking the mutated *CTNNB1* gene copy (Fig. 2A). In contrast, isogenic cells lacking the wild-type gene copy of *CTNNB1* (ΔS45/–) showed about similar sensitivity to TTK inhibitors as parental HCT 116 cells (Fig. 2A). The doubling times of the HCT 116 (ΔS45/+, parental), (ΔS45/–) and (–/+) variants were 21, 22, and 29 hours, respectively, which is consistent with the lower ability of the (–/+) variant to form colonies, as reported earlier (26).

The other TTK inhibitors also showed less activity in proliferation assays with the isogenic cell line lacking the mutant *CTNNB1* gene copy (Fig. 2B and C; Supplementary Fig. S4; Supplementary Table S3B). The differences in sensitivity were of similar magnitude as those between wild-type and mutant lines in the 66 cell line panel, with compound 5 showing the strongest targeting. There the ΔpIC_{50} between parental (ΔS45/+) and the isogenic (–/+) cell line was 0.58 (Supplementary Table S3B).

To study the mechanism of the interaction between mutant *CTNNB1* and TTK inhibition, we studied the effect of NTRC 0066-0 on cytoplasm-to-nuclear translocation of β-catenin and expression of the Wnt pathway target gene *Axin2* by Q-PCR in the HCT116 parental and the isogenic (–/+) cell line, and the lung carcinoma cell line A427, which is homozygous mutant for *CTNNB1* (Table 1). Activation of the Wnt pathway, as determined by *Axin2* gene expression, correlated with *CTNNB1*-mutant status, but there was no direct effect of TTK inhibition (Supplementary Fig. S3A), indicating that TTK inhibitors do not directly interfere with the Wnt pathway.

Validation using functional assay data and GSA

To further explore the mechanism of the association between *CTNNB1* mutation and sensitivity to TTK inhibition, we determined β-catenin pathway activity in sensitive cell lines from the Oncolines panel. First, we ascertained by Western blot analysis and qPCR that the *CTNNB1*-mutant cell lines in the Oncolines panel contain nuclear β-catenin and express *Axin2*. This was indeed the case, as already had been described in the literature (Supplementary Fig. S3B; refs. 26, 32, 36). Second, to independently study if TTK inhibitor sensitivity is linked to β-catenin transcriptional activity, we used a literature dataset of 85 cancer cell lines labeled "active" or "inactive" in a β-catenin/TCF4 reporter assay (37). Of this dataset, six cell lines with validated reporter assay activity are also part of the Oncolines cell panel (A-549, COLO 205, DLD-1, SW48, and SW480), as well as seven "inactive" cell lines (AsPC-1, BxPC-3, MIA Paca-2, NCI-H82, OVCAR-3, RKO, and TT). Comparison of inhibitor responses between these two groups indicates that the cell lines active in the β-catenin/TCF4 reporter assay are also significantly more sensitive to TTK inhibitors ($P \leq 0.05$, Student t test and Wilcoxon test, Supplementary Table S2C). Thus, these literature data validate the biomarker and suggest that TTK inhibitor sensitivity is linked to β-catenin transcriptional activity.

As markers for drug sensitivity can also be found from mRNA expression profiles (29) we next used GSA on the cell panel data. We generated a list of genes of which high expression correlates with sensitivity to NTRC 0066-0, using gene expression information from 57 cell lines. Because many more lines than the *CTNNB1* mutants are analyzed, GSA provides an orthogonal analysis of the biomarker's mechanism. We investigated if genes related to Wnt/β-catenin signaling were significantly

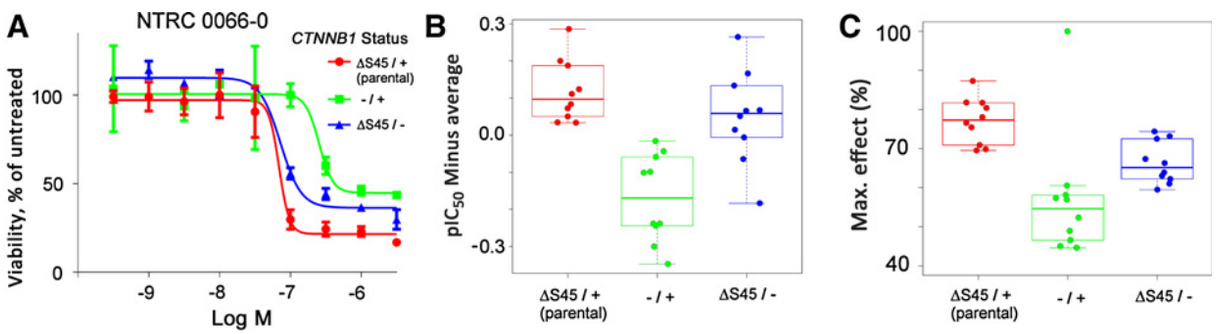

Figure 2.

Validation of mutant *CTNNB1* as drug sensitivity biomarker for TTK inhibitors using isogenic cell lines. **A,** Representative dose–response curves of NTRC 0066-0 in proliferation assays with parental HCT 116 cells (ΔS45/+, red) and an isogenic cell line lacking the mutated *CTNNB1* gene copy (–/+, green) or the wild-type gene copy (ΔS45/–, blue). **B** and **C**, Box plot showing distribution of relative potencies (pIC_{50}s; **B**) and maximum percentage effect (efficacy; **C**) of 10 TTK inhibitors in proliferation assays in the three isogenic cell lines. Every dot represents 1 of the 10 TTK reference inhibitors. For data in **B** and **C**, averages of three independent experiments are used. pIC_{50} differences compared with the average over the three isogenic variants are shown. Maximum effect is defined as the difference between top and bottom of dose–response curves. A Wilcoxon rank sum test on the combined inhibitor data shows that TTK inhibitors have significantly more potent pIC_{50} and higher maximum effects in both ΔS45/+ and ΔS45/– cells compared with nonmutant (+/–; $P < 0.01$).

overrepresented at the top of the gene list. This is the case with genes that are upregulated after transfection with active *CTNNB1*, as well as genes that are downregulated after knockdown of *CTNNB1* (for references and gene set names, see Supplementary Table S4A). Probing with mechanism-based gene sets reveals that TTK inhibitor–sensitive cell lines have active MYC and FOXM1 signaling. Notably, FOXM1 is part of the β-catenin transcriptional complex but also a regulator of mitosis (refs. 38, 39; Supplementary Table S4B). Gene sets related to β-catenin regulation were not significant, probably because this is not controlled by mRNA expression but by protein stability (Supplementary Table S4B). Thus, GSA corroborates that TTK inhibitor–sensitive cells have an active β-catenin pathway.

In vivo efficacy and potential therapeutic relevance in human cancers

We have previously shown that 20 mg/kg NTRC 0066-0 given orally every other day reduces tumor growth in a mouse xenograft model of the human TNBC cell line MDA-MB-231 by 61% (8). To investigate whether TTK inhibitor therapy is more efficacious in the context of mutated *CTNNB1*, we attempted to set up an *in vivo* model using HCT116 and its isogenic variants. However, this was hampered by the appearance of ulcerations in xenografts of the untreated HCT 116 parental cell line, preventing further studies for ethical reasons. As an alternative, we investigated whether the superior *in vitro* sensitivity of the cell line A427 also translates to an *in vivo* situation. An *in vivo* efficacy study was therefore carried out with a xenograft model of the lung carcinoma cell line A427, which is homozygous mutant for the *CTNNB1* gene (36). In this model, NTRC 0066-0 reduced tumor growth by 90% (Fig. 3A). In a parallel study, NTRC 0066-0 reduced tumor growth in the MDA-MB-231 model by 70% (Fig. 3B). At the doses used, there was no significant effect on body weight of the mice in both models (Fig. 3C), in agreement with our previous study (8).

Frequency of *CTNNB1* mutations in patients

Our studies suggest that TTK inhibitors may be in particular effective in cancers characterized by mutations in the *CTNNB1* gene. To determine the potential relevance for targeted therapy, we analyzed The Cancer Genome Atlas (TCGA) (http://www.cancergenome.nih.gov) for the occurrence of *CTNNB1* mutations in different indications. *CTNNB1* gene mutations were filtered for mutations corresponding to the four regulatory phosphorylation sites in β-catenin encoded by exon 3. These mutations are frequently detected in tumor samples from endometrial cancer and hepatocellular carcinoma (HCC; Table 2), which both have a high incidence. Whereas endometrial cancer is often treated with surgery, there is a high medical need of more effective therapies for HCC. Adrenal cancer and medulloblastoma are cancers with relatively low incidence and relatively high expression (>10%) of mutant *CTNNB1* (Table 2). Whereas mutations in *CTNNB1* are detected at a much lower frequency in prostate and lung, both cancers have a high incidence (Table 2).

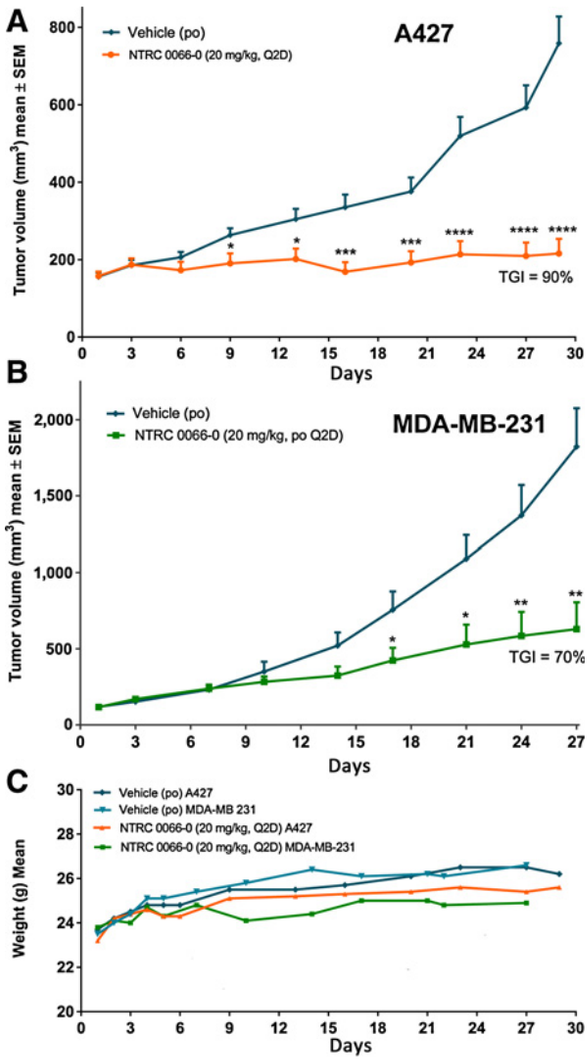

Figure 3.

Inhibition of tumor growth in two mouse xenograft models, with or without mutation in the *CTNNB1* gene. Mice were treated with vehicle or 20 mg/kg NTRC 0066-0 administered orally every other day ($n = 10$ mice per treatment group, mean and SEM). Unpaired *t* test: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$. **A,** Time course of tumor growth of the A427 xenograft model, homozygous CTNNB1-mutant lung carcinoma cell line. **B,** Time course of tumor growth of orthotopic xenograft model of the triple-negative breast cancer cell line MDA-MB-231, which is homozygous wild-type for the *CTNNB1* gene. **C,** Time course of animal body weight gain of A427 and MDA-MB-231 model.

Discussion

Inhibition of the spindle assembly checkpoint kinase TTK is a relatively new, and still experimental therapeutic approach to selectively target proliferating cancer cells. Supported by positive results from efficacy studies in preclinical models (8, 12, 14), TTK inhibitors have been positioned as potential new drugs in combination with taxane chemotherapy in TNBC (8, 12). Our unbiased cell panel screen shows that TTK inhibitors are indeed very effective in TNBC cell lines (8, 30), but do not confirm earlier results of *TP53* or *PTEN* targeting (16, 17). Instead, our unbiased cell panel profiling indicates that TTK inhibitors may be more effective in cancers characterized by activating mutations in the *CTNNB1* gene.

The distribution of *CTNNB1*-mutant cell lines in the waterfall plot (Fig. 1A) indicates that mutant *CTNNB1* is not the sole determinant of TTK inhibitor sensitivity. Recently, we showed by time-lapse image analysis of six cancer cell lines from the 66 cancer cell line panel, that chromosomally stable aneuploid cell lines are relatively more sensitive to TTK inhibition than

Table 2. Top 10 cancers expressing mutant *CTNNB1* and cancer incidence in the United States

Cancer	Mutant *CTNNB1*[a]	Incidence[b]	Deaths[b]	Current therapy
Endometrium (uterine corpus)	30%	61,380	10,920	Mainly surgery
Hepatocellular carcinoma	27%	40,710	28,920	Sorafenib for nonresectable hepatocellular carcinoma
Adrenal cancer	17%	200	—	Mitotane (blocks hormone production)
Medulloblastoma	13%	400	—	Chemotherapy
Gastric	8%	28,000	10,960	Herceptin
Melanoma	7%	87,110	9,730	Targeted therapy and immunotherapy
Colorectal	6%	135,430	50,260	Irinotecan; stage IV, bevacuzimab
Lung	4%	222,500	155,870	Chemotherapy, targeted therapies
Prostate	3%	161,360	26,730	Hormone therapy
Pancreatic	3%	53,670	43,090	Gemcitabine; 5-fluorouracil

[a]Frequency of total genomic changes from TCGA. Medulloblastoma data from International Cancer Genome Consortium (ICGC). As reported on cBioPortal April 13th, 2017.

[b]Incidence and number of deaths from American Cancer Society website, estimates 2017, April 14, 2017. Except medulloblastoma: American Brain Tumor Association. For adrenal cancer and medulloblastoma, death statistics are not exact.

intrinsically chromosomally unstable cell lines (30). Treatment of sensitive cell lines with TTK inhibitors increased the percentage of cells undergoing mis-segregations, which was already maximal in resistant cell lines. This suggests that TTK-sensitive lines are more dependent on an active SAC (30). In that study, we used the near-diploid cell line HCT116 that is chromosomally stable and *CTNNB1* mutant. Two other chromosomally stable cell lines studied, LoVo and A-172 (30), are wild-type for *CTNNB1*. Interestingly, the *CTNNB1*-mutant lines SW48 and LS 174T are also near-diploid and chromosomally stable, whereas A-427 is near-triploid and chromosomally stable (40, 41). Thus, the *CTNNB1*-mutant lines have a similar stable karyotype as found earlier in relatively sensitive cells (30). The mechanistic link between *CTNNB1* mutation and sensitivity to TTK inhibition is probably related to β-catenin signaling, as indicated by the results from the functional assays and gene set analysis. Whereas β-catenin has a well-known role in cell adhesion and regulation of gene expression (31), β-catenin also contributes to mitosis in the establishment of a bipolar mitotic spindle (42). Activation of Wnt signaling by *CTNNB1* or *APC* mutations has been reported to increase chromosomal instability and cause aneuploidy (43–45). TTK, on the other hand, has been shown to protect aneuploid cells from mitotic catastrophe (4, 42). Interestingly, both β-catenin signaling and mitosis are mediated by *FOXM1*, which appeared from the GSA (38, 39). Like TTK expression, β-catenin phosphorylation status fluctuates with the cell cycle (47). β-catenin is maximally active during G_1–S and disappears at G_2–M under the influence of its transcriptional product conductin/Axin2 (47). This is probably because maintaining Wnt-dependent transcription during mitosis is toxic to cells (47). TTK inhibitors could disrupt the cycling process because they reduce time in mitosis by overriding the SAC (8, 30), leaving β-catenin–mutant cells no time to critically downregulate Wnt signaling during mitosis (Fig. 4).

On the basis of these data, we hypothesize that the synthetic lethal effect between TTK inhibition and *CTNNB1* mutation arises because disregulated β-catenin signaling in mutant cells makes them more sensitive to mitotic catastrophe. Cells that survive the mutation therefore possess a chromosomally stable karyotype maintained by a strong mitotic checkpoint activation through *FOXM1* and cell-cycle control through conductin/Axin2. As a result, *CTNNB1*-mutant cells particularly depend on the SAC and thereby TTK, making them vulnerable to TTK inhibitors (Fig. 4).

The reason why *CTNNB1* mutation shows more significant association than *APC* mutation could lie in the fact that β-catenin engages in a direct feedback regulation with conductin/Axin2 (47) and *FOXM1* (38), whereas *APC* can also interfere with mitosis through a β-catenin–independent process called Wnt/STOP (48). The mechanistic basis of the association between activated β-catenin and cellular response to TTK inhibitors however requires further study.

Although our cell panel profiling results do not confirm earlier work on *TP53* or *PTEN* targeting of TTK inhibitors, they do confirm that TTK inhibitors are more active in TNBC cell lines than in cell lines from other cancer types. For instance, the average IC_{50} of NTRC 0066-0 on four TNBC cell lines (BT-20, BT-549, Hs 578T, MDA-MB-231) is 49 nmol/L, whereas the average IC_{50} for all other cell lines in the panel is 99 nmol/L (and 123 nmol/L for all non-TNBC breast cancer lines). Although *CTNNB1* mutations are rare in TNBC, multiple studies have confirmed that the Wnt/catenin pathway is often activated in TNBC, and is indicative for prognosis (49, 50). Targeting β-catenin in TNBC cell lines leads to significant growth reduction (51). A potential pathway activator could be the Wnt ligand Wnt10b, which is involved in mammary development and plays an important role in development of TNBC tumors (52). Therefore, the preferential targeting of TNBC lines and *CTNNB1*-mutant cell lines by TTK inhibitors might be related.

We propose that mutations in sequences that regulate the degradation of β-catenin can be used as prognostic drug response biomarkers to select patient populations that are most likely to respond to TTK inhibitor therapy (53), and suggest new potential applications for TTK inhibitors, such as endometrial cancer and HCC. Notably, high *TTK* expression correlates with poor prognosis in HCC (15). Our study provides an additional argument to pursue the development of TTK inhibitor therapy for HCC and other diseases driven or dependent on activated β-catenin. During the course of our analysis, we discovered however that almost no *CTNNB1* hotspot mutant cell lines are available from these cancers. The only exception is SNU-398, a liver line that expresses Hepatitis B viral sequences. Thus, there is an urgent need for more *CTNNB1*-mutant cell line models for liver and endometrial cancers.

Interestingly, β-catenin signaling has also been implicated in intrinsic resistance against immunotherapy in melanoma by regulation of T-cell infiltration (54). In a recent article, Mason and colleagues (13) showed that TTK inhibitor therapy in combination with anti-programmed cell death 1 (PD-1) antibody can induce tumor regression in a syngeneic mouse model of human cancer. One possible explanation of this response, raised by the authors, is the induction of genomic instability and apoptotic cell

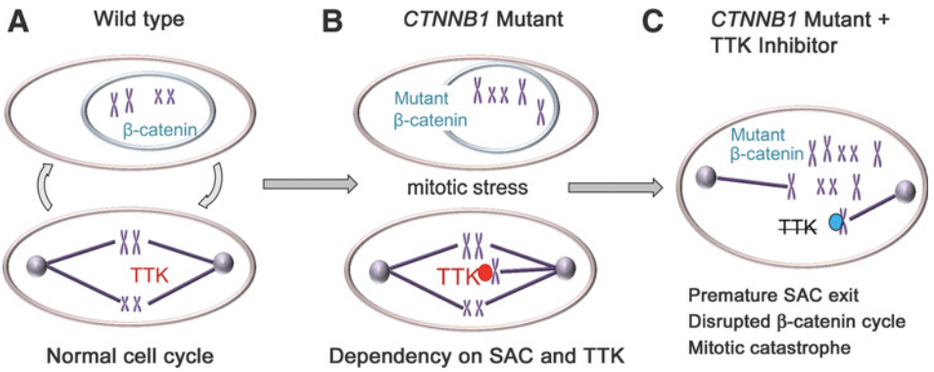

Figure 4.
Model of the interaction between β-catenin and TTK. **A,** During the normal cell cycle, β-catenin is expressed in G_1–S phase, where it regulates transcription, and TTK is expressed in the G_2–M phase where it regulates the spindle assembly checkpoint. **B,** β-catenin mutation is known to lead to chromosomal instability (CIN) and mitotic stress, requiring prolonged SAC signaling to survive mitosis, which is synonymous with dependency on TTK signaling. **C,** Inhibition of TTK abrogates the SAC and interrupts the cycle of β-catenin expression. This leads to more severe aneuploidy and mitotic catastrophe.

death by TTK inhibition, which may promote tumor immunity. Alternatively, or in addition, as suggested by our work, TTK inhibition may directly regulate T-cell response by affecting β-catenin–regulated immune response.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: G.J.R. Zaman, J.A.D.M. de Roos, R.C. Buijsman, J.C.M. Uitdehaag

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J.A.D.M. de Roos, M.A.A. Libouban, M.B.W. Prinsen, J. de Man

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): G.J.R. Zaman, J.A.D.M. de Roos, M.A.A. Libouban, M.B.W. Prinsen, R.C. Buijsman, J.C.M. Uitdehaag

Writing, review, and/or revision of the manuscript: G.J.R. Zaman, J.C.M. Uitdehaag

Study supervision: G.J.R. Zaman

Acknowledgments

We would like to thank Jelle Dylus for cell line proliferation experiments and Jeffrey Kooijman for bioinformatics analyses.

Grant Support

This work was supported by the European Commission FP7 Marie Curie Action "PloidyNet" (607722; to G.J.R. Zaman).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received April 19, 2017; revised July 12, 2017; accepted July 20, 2017; published OnlineFirst July 27, 2017.

References

1. Higgins MJ, Baselga J. Targeted therapies for breast cancer. J Clin Invest 2011;121:3797–803.
2. Bollag G, Hirth P, Tsai J, Zhang J, Ibrahim PN, Cho H, et al. Clinical efficacy of a RAF inhibitor needs broad target blockade in B-RAF-mutant melanoma. Nature 2010;467:596–9.
3. Liu X, Winey M. The MPS1 family of protein kinases. Annu Rev Biochem 2012;81:561–85.
4. Janssen A, Kops GJPL, Medema RH. Elevating the frequency of chromosome mis-segregation as a strategy to kill tumor cells. Proc Natl Acad Sci U S A 2009;206:19108–13.
5. Colombo R, Caldarelli M, Mennecozzi M, Giorgini ML, Sola F, Cappella P, et al. Targeting the mitotic checkpoint for cancer therapy with NMS-P715, an inhibitor of MPS1 kinase. Cancer Res 2010;70:10255–64.
6. Kwiatkowski N, Jelluma N, Filippakopoulos P, Soundararajan M, Manak MS, Kwon M, et al. Small-molecule kinase inhibitors provide insight into Mps1 cell cycle function. Nat Chem Biol 2010;6:359–68.
7. Jemaà M, Galluzzi L, Kepp O, Senovilla L, Brands M, Boemer U, et al. Characterization of novel MPS1 inhibitors with preclinical anticancer activity. Cell Death Differ 2013;20:1532–45.
8. Maia AR, de Man J, Boon U, Janssen A, Song JY, Omerzu M, et al. Inhibition of the spindle assembly checkpoint kinase TTK enhances the efficacy of docetaxel in a triple-negative breast cancer model. Ann Oncol 2015; 26:2180–92.
9. Tardif KD, Rogers A, Cassiano J, Roth BL, Cimbora DM, McKinnon R, et al. Characterization of the cellular and antitumor effects of MPI-0479605, a small-molecule inhibitor of the mitotic kinase Mps1. Mol Cancer Ther 2011;10:2267–75.
10. Kusakabe K, Ide N, Daigo Y, Itoh T, Yamamoto T, Hashizume H, et al. Discovery of imidazo[1,2-b]pyridazine derivatives: selective and orally available Mps1 (TTK) kinase inhibitors exhibiting remarkable antiproliferative activity. J Med Chem 2015;58:1760–75.
11. Martinez R, Blasina A, Hallin JF, Hu W, Rymer I, Fan J, et al. Mitotic checkpoint kinase Mps1 has a role in normal physiology which impacts clinical utility. PLOS One 2015;10:e0138616.
12. Wengner AM, Siemeister G, Koppitz M, Schulze V, Kosemund D, Klar U, et al. Novel Mps1 kinase inhibitors with potent antitumor activity. Mol Cancer Ther 2016;15:583–92.
13. Mason JM, Wei X, Fletcher GC, Kiarash R, Brokx R, Hodgson R, et al. Functional characterization of CFI-402257, a potent and selective Mps1/TTK kinase inhibitor, for the treatment of cancer. Proc Natl Acad Sci U S A 2017;114;3127–32.
14. Maire V, Baldeyron C, Richardson M, Tesson B, Vincent-Salomon A, Gravier E, et al. TTK/hMPS1 is an attractive therapeutic target for triple-negative breast cancer. PLoS One 2013;8:e63712.
15. Liu X, Liao W, Yuan Q, Ou Y, Huang J. TTK activates Akt and promotes proliferation and migration of hepatocellular carcinoma cells. Oncotarget 2015;6:34309–20.
16. Brough R, Frankum JR, Sims D, Mackay A, Mendes-Pereira AM, Bajrami I, et al. Functional viability profiles of breast cancer. Cancer Discov 2011; 1:260–73.
17. Győrffy B, Bottai G, Lehmann-Che J, Kéri G, Őrfi L, Iwamoto T, et al. TP53 mutation-correlated genes predict the risk of tumor relapse and identify MPS1 as a potential therapeutic kinase in TP53-mutated breast cancers. Mol Oncol 2014;8:509–14.
18. Uitdehaag JCM, de Roos JADM, van Doornmalen AM, Prinsen MB, de Man J, Tanizawa Y, et al. Comparison of the cancer gene targeting and biochemical selectivities of all targeted kinase inhibitors approved for clinical use. PLoS One 2014;9:e92146.

19. Uitdehaag JC, de Roos JA, Prinsen MB, Willemsen-Seegers N, de Vetter JR, Dylus J, et al. Cell panel profiling reveals conserved therapeutic clusters and differentiates the mechanism of action of different PI3K/mTOR, Aurora kinase and EZH2 inhibitors. Mol Cancer Ther 2016;15: 3097–109.
20. Schulze V, Mais FJ, inventors, Bayer Pharma Aktiengesellschaft, assignee. Method for preparing substituted triazolopyridines. Patent WO2014/ 009219 A1. 2014 Jan 16.
21. Koppitz M, Klar U, Wengner AM, Neuhaus R, Siemeister G, Brüning M, et al. inventors; Bayer Intellectual Property Gmbh, assignee. Substituted imidazopyridazines. Patent WO2014/131739 A2. 2014 Sep 4.
22. Koppitz M, Klar U, Jautelat R, Kosemund D, Bohlmann R, Bader B, et al. inventors; Bayer Intellectual Property LLC, assignee. Imidazopyrazines for use as Mps1 and TKK inhibitors in the treatment hyperproliferative disorders. Patent WO2012/080229 A1. 2012 Jun 21.
23. Kumar DV, Hoarau C, Bursavich M, Slattum P, Gerrish D, Yager K, et al. Lead optimization of purine based orally bioavailable Mps1 (TTK) inhibitors. Bioorg Med Chem Lett 2012;22:4377–85.
24. de Man AP, Buijsman RC, Sterrenburg JG, Uitdehaag JC, de Wit JJ, Zaman GJ, inventors; Netherlands Translational Research Center B.V., assignee. (5,6-dihydro)pyrimido[4,5-e]indolizines. Patent WO 2015/155042 A1. 2015 Oct 15.
25. Forbes SA, Bhamra G, Bamford S, Dawson E, Kok C, Clements J, et al. The catalogue of somatic mutations in cancer (COSMIC). Curr Protoc Hum Genet 2008;Chapter 10:Unit 10.11.
26. Chan TA, Wang Z, Dang LH, Vogelstein B, Kinzler KW. Targeted inactivation of CTNNB1 reveals unexpected effects of beta-catenin mutation. Proc Natl Acad Sci U S A 2002;99:8265–70.
27. R Development Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2008.
28. Barretina J, Caponigro G, Stransky N, Venkatesan K, Margolin AA, Kim S, et al. The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. Nature 2012;483;603–7.
29. Väremo L, Nielsen J, Nookaew I. Enriching the gene set analysis of genome-wide data by incorporating directionality of gene expression and combining statistical hypotheses and methods. Nucleic Acids Res 2013;41;4378–91.
30. Libouban MA, de Roos JA, Uitdehaag JC, Willemsen-Seegers N, Mainardi S, Dylus J, et al. Stable aneuploid cells are more sensitive to TTK inhibition than chromosomally unstable cell lines. Oncotarget 2017;8; 38309–25.
31. Logan CY, Nusse R. The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol 2004;20:781–810.
32. Morin PJ, Sparks AB, Korinek V, Barker N, Clevers H, Vogelstein B, et al. Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. Science 1997;275:1787–90.
33. Uitdehaag JC, de Man J, Willemsen-Seegers N, Prinsen MB, Libouban MA, Sterrenburg JG, et al. Target residence time-guided optimization on TTK kinase results in inhibitors with potent anti-proliferative activity. J Mol Biol 2017;429;2211–30.
34. Colombo R, Burbridge M, Rodriguez M, Cantero F, Caldarelli M, Giorgini ML, et al. Preclinical characterization of the novel TTK kinase inhibitor S81694 for the treatment of triple negative breast cancer. Cancer Res 2015;75:15.
35. Faisal A, Mak GW, Gurden MD, Xavier CP, Anderhub SJ, Innocenti P, et al. Characterisation of CCT271850, a selective, oral and potent Mps1 inhibitor, used to directly measure in vivo Mps1 inhibition vs therapeutic efficacy. Br J Cancer 2017;116;1166–76.
36. Sunaga N, Kohno T, Kolligs FT, Fearon ER, Saito R, Yokota J. Constitutive activation of the Wint signaling pathway by CTNNB1 (β-catenin) mutations in a subset of human lung adenocarcinoma. Genes Chromosomes Cancer 2001;30:316–21.
37. Rosenbluh J, Nijhawan D, Cox AG, Li X, Neal JT, Schafer EJ, et al. β-Catenin-driven cancers require a YAP1 transcriptional complex for survival and tumorigenesis. Cell 2013;51;1457–73.
38. Zhang N, Wei P, Gong A, Wen-Tai C, Hsueh-Te L, Colman H, et al. FoxM1 promotes *β*-catenin nuclear localization and controls Wnt target gene expression and glioma tumorigenesis. Cancer Cell 2015;20;427–42.
39. Laoukili J, Kooistra MRH, Brás A, Kauw J, Kerkhoven RM, Morrison A, et al. FoxM1 is required for execution of the mitotic programme and chromosome stability. Nat Cell Biol 2005;7;126–36.
40. Knutsen T, Padilla-Nash HM, Wangsa D, Barenboim-Stapleton L, Camps J, McNeil N, et al. Definitive molecular cytogenetic characterization of 15 colorectal cancer cell lines. Genes Chromosomes Cancer 2010;49;204–23.
41. Speicher MR, Petersen S, Uhrig S, Jentsch I, Fauth C, Eils R, et al. Analysis of chromosomal alterations in non-small cell lung cancer by multiplex FISH, comparative genomic hybridization and multicolor bar coding. Lab Invest 2000;80;1031–41.
42. Kaplan DD, Meigs TE, Kelly P, Casey PJ. Identification of a role for β-catenin in the establishment of a bipolar mitotic spindle. J Biol Chem 2004; 279:10829–32.
43. Fodde R, Kuipers J, Rosenberg C, Smits R, Kielman M, Gaspar C, et al. Mutations in the APC tumour suppressor gene cause chromosomal instability. Nat Cell Biol 2001;3:433–8.
44. Aoki K, Aoki M, Sugai M, Harada N, Miyoshi H, Tsukamoto T, et al. Chromosomal instability by β-catenin/TCF transcription in APC or β-catenin mutant cells. Oncogene 2007;26:3511–20.
45. Hadjihannas MV, Brückner M, Jerchow B, Birchmeier W, Dietmaier W, Behrens J. Aberrant Wnt/β-catenin signaling can induce chromosomal instability in colon cancer. Proc Natl Acad Sci U S A 2006;103;10747–52.
46. Daniel J, Coulter J, Woo JH, Wilsbach K, Gabrielson E. High levels of the Mps1 checkpoint protein are protective of aneuploidy in breast cancer cells. Proc Natl Acad Sci U S A 2011;108;5384–9.
47. Hadjihannas MV, Bernkopf DB, Bruckner M, Behrens J. Cell cycle control of Wnt/*β*-catenin signaling by conductin/axin2 through CDC20. EMBO Rep 2012;13:347–54.
48. Stolz A, Bastians H. Fresh WNT into the regulation of mitosis. Cell Cycle 2015;14;2566–70.
49. Khramtsov AI, Khramtsova GF, Tretiakova M, Huo D, Olopade OI, Goss KH. Wnt/β-catenin activation is enriched in basal-like breast cancers and predicts poor outcome. Am J Pathol 2010;176:2911–20.
50. Xu J, Prosperi JR, Choudhury N, Olopade OI, Goss KH. β-catenin is required for the tumorigenic behavior of triple-negative breast cancer. PLoS ONE 2015;10:e0117097.
51. Geyer FC, Lacroix-Triki M, Savage K, Arnedos M, Lambros MB, MacKay A, et al. β-catenin pathway activation in breast cancer is associated with triple-negative phenotype but not with CTNNB1 mutation. Mod Pathol 2011;24:209–31.
52. Wend P, Runke S, Wend K, Anchondo B, Yesayan M, Jardon M, et al. WNT10B/β-catenin signalling induces HMGA2 and proliferation in metastatic triple-negative breast cancer. EMBO Mol Med 2013;5:264–79.
53. de Roos JA, Uitdehaag JC, de Man AP, Buijsman RC, Zaman GJR, inventors; Netherlands Translational Research Center B.V., assignee. Prognostic biomarkers for TTK inhibitor chemotherapy. Patent WO 2016/166255 A1. 2016 Oct 20.
54. Spranger S, Bao R, Gajewski TF. Melanoma-intrinsic β-catenin signalling prevents anti-tumor immunity. Nature 2015;523:231–5.