HER2-Mediated Internalization of Cytotoxic Agents in ERBB2 Amplified or Mutant Lung Cancers

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Amplification of and oncogenic mutations in ERBB2, the gene encoding the HER2 receptor tyrosine kinase, promote receptor hyperactivation and tumor growth. Here we demonstrate that HER2 ubiquitination and internalization, rather than its overexpression, are key mechanisms underlying endocytosis and consequent efficacy of the anti-HER2 antibody–drug conjugates (ADC) ado-trastuzumab emtansine (T-DM1) and trastuzumab deruxtecan (T-DXd) in lung cancer cell lines and patient-derived xenograft models. These data translated into a 51% response rate in a clinical trial of T-DM1 in 49 patients with ERBB2-amplified or -mutant lung cancers. We show that cotreatment with irreversible pan-HER inhibitors enhances receptor ubiquitination and consequent ADC internalization and efficacy. We also demonstrate that ADC switching to T-DXd, which harbors a different cytotoxic payload, achieves durable responses in a patient with lung cancer and corresponding xenograft model developing resistance to T-DM1. Our findings may help guide future clinical trials and expand the field of ADC as cancer therapy.

SIGNIFICANCE: T-DM1 is clinically effective in lung cancers with amplification of or mutations in ERBB2. This activity is enhanced by cotreatment with irreversible pan-HER inhibitors, or ADC switching to T-DXd. These results may help address unmet needs of patients with HER2-activated tumors and no approved targeted therapy.

See related commentary by Rolfo and Russo, p. 643.
Li et al. (2020) demonstrated that activated HER2, regardless of its oncogenic potential or the addiction status of the cancer cell to its downstream signaling pathways, can serve as a vehicle to funnel potent chemotherapeutic agents into lung tumors. Integrating parallel laboratory and clinical data on the mechanisms of response, we also propose two different strategies to improve the efficacy of anti-HER2 ADCs: co-treatment with irreversible pan-HER inhibitors that enhance receptor ubiquitination and internalization of ADCs or switching to an ADC bearing a different cytotoxic payload.

RESULTS

HER2 Mutations Increase Receptor Internalization and T-DM1 Activity

We hypothesized that ERB2-amplified or -mutated tumors have exquisite susceptibility to HER2 ADCs on the basis of high-rate receptor internalization and trafficking, regardless of their intrinsic dependence on HER2 signaling for cell growth and/or survival. To test whether the presence of an activating mutation influences HER2 internalization rate, affecting in turn the internalization rate of ADC–HER2 complexes, we established isogenic models using a nontransformed breast cancer cell (MCF10A) and a lung cancer cell line (NCI-H2030) expressing either V5-tagged wild-type (WT) or mutant (S310F or L755S) HER2 or empty vector (EV) as control. We used these models to quantitate the internalization rate of T-DM1 linked to a pH-sensitive dye (pHrodo-T-DM1) that becomes fluorescent only at low pH, providing a positive indication of ADC–HER2 endocytosis. Albeit with some variations in the kinetics, we consistently observed that cells expressing either S310F or L755S mutants internalize more T-DM1 than WT cells in both cell models (Fig. 1A–D). In particular, MCF10A cells expressing the HER2S310F mutant show the highest pHrodo-T-DM1 signal at earlier time points and morphologic changes at later time points (Fig. 1A and B), compatible with the onset of cell death, as confirmed by increased PARP cleavage (Fig. 1E). In both cell lines, expressing comparable WT or mutant HER2, receptor levels were reduced upon T-DM1 treatment, likely due to internalization and subsequent degradation (Fig. 1E and F).

On the basis of these data, we posited that T-DM1 can accumulate in both mutant and amplified lung tumors. To evaluate this in the clinical setting, we performed 89Zr-trastuzumab PET/CT functional imaging in patients with lung cancer bearing HER2 alterations. We found that 89Zr-trastuzumab can accumulate in both ERB2-amplified and ERB2-mutant tumors (Fig. 1G, H).

We then tested the antitumoral activity of T-DM1 in different patient-derived xenografts (PDX) established from patients with lung cancer with tumors bearing ERB2 mutations or amplification. Treatment with T-DM1 resulted in durable responses in both HER2-mutant (YVMA insertion) and -amplified or -mutated lung cancers (Table 1) were treated with T-DM1, and responses were measured by RECIST version 1.1 and/or modified PET Response Criteria in Solid Tumors (PERCIST). Modified PERCIST, recently used to assess antitumor activity of new cancer therapies in clinical trials (13, 20, 21), was employed to capture RECIST nonmeasurable disease. The

CI

Clinical Activity of T-DM1 in Patients with HER2-Activated Lung Cancer

The above data provided the rationale for a dedicated analysis of the clinical activity of T-DM1 in patients with ERB2-amplified or -mutant lung cancers within the context of a histology-agnostic phase II basket trial (NCT02675829; Supplementary Fig. S2). In total, 49 patients (18/49 patients were part of the previously reported ERB2-mutant cohort of this clinical trial; ref. 19) with ERB2-mutant and/or -amplified lung cancer (Table 1) were treated with T-DM1, and responses were measured by RECIST version 1.1 and/or modified PET Response Criteria in Solid Tumors (PERCIST).
Antitumoral Activity of Anti-HER2 ADCs in Lung Cancer

**A**

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**B**

![Graph showing trafficking index](image)

**C**

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**D**

![Graph showing trafficking index](image)

**E**

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<th>L755S</th>
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**F**

![Graph showing trafficking index](image)

**G**

**ERBB2-amplified patient**

FDG PET/CT

89Zr-trastuzumab PET/CT

**H**

**ERBB2-mutant patient**

FDG PET/CT

89Zr-trastuzumab PET/CT

**I**

**ERBB2 mutation YVMA**

Tumor volume (mm^3 ± SEM) vs. Days of treatment

**J**

**ERBB2 mutation S310F and amplification**

Tumor volume (mm^3 ± SEM) vs. Days of treatment
The best overall response rate (ORR) by either RECIST or modified PERCIST for ERBB2-amplified/mutant patients was 51% [25/49; 95% confidence interval (CI) 36–66; Fig. 2A and B], with a median progression-free survival (PFS) of 5 months (95% CI, 3.5–5.9; Fig. 2C). In the subset of ERBB2-mutant and/or -amplified patients with RECIST evaluable disease, the ORR by RECIST was 31% (13/42; 95% CI, 18–47; Supplementary Fig. S3A and S3B), whereas in the subset of ERBB2-mutant and/or -amplified patients with PERCIST evaluable disease, the ORR by modified PERCIST was 64% (14/22; 95% CI, 41–83; Supplementary Fig. S3C and S3D). Except for 1 case (2%) of grade 3 febrile neutropenia, treatment was well tolerated (Table 2).

In accordance with our preclinical data (Fig. 1), we did not observe appreciable differences in response when we stratified patients according to ERBB2 genetic status: The best ORR by either RECIST or modified PERCIST was 55% (6/11; 95% CI, 23%–83%) for ERBB2-amplified patients, 50% (14/28; 95% CI, 31–69) for ERBB2-mutant patients and 50% (5/10; 95% CI, 19–81) for concurrently ERBB2-mutant and -amplified patients (Fig. 2A). These data strongly suggest that a response to T-DM1 can be achieved to the same extent in lung cancers with either amplified or mutant ERBB2.

ERBB2 copy number obtained by next-generation sequencing (NGS) using the Fraction and Allele-Specific Copy Number Estimates from Tumor Sequencing (FACETS) algorithm (22) correlated with HER2 status as assessed by FISH and IHC (Supplementary Fig. S3E–S3G, Supplementary Table S1). A trend of association between the degree of ERBB2 amplification by FACETS with patient response to T-DM1 therapy was observed, albeit not reaching statistical significance (Supplementary Fig. S3H). There were 6 patients with ERBB2-amplified lung cancer and concurrent EGFR mutation who had progressed on a prior EGFR inhibitor, and 2 of these patients responded to T-DM1 (Supplementary Table S1).

**Irreversible HER Kinase Inhibitors Enhance HER2 Internalization and T-DM1 Activity**

Despite the promising clinical activity observed with T-DM1 in ERBB2-mutant/amplified lung cancers, some patients’ tumors were intrinsically refractory to T-DM1 and some responses were short-lived. We then sought strategies to enhance or prolong the duration of the responses to T-DM1 in lung cancers. The irreversible pan-HER kinase inhibitors neratinib and afatinib are approved agents for HER2-overexpressing breast cancer and EGFR-mutant lung cancer, respectively. In addition to their strong activity in inhibiting HER2 phosphorylation, they are thought to induce receptor polyubiquitination and internalization, likely due to hindrance of HSP90 binding (23–25). We thus tested whether these findings could be translated to ERBB2-amplified or -mutant lung cancers. Cotreatment of Calu-3 (ERBB2 amplified) and LUAD-10 (patient-derived ERBB2L755P mutant) lung cancer cells with the irreversible inhibitor neratinib markedly increased T-DM1 internalization, as quantitated by pHrodo-T-DM1 live imaging (Fig. 3A and B). In contrast, cotreatment with the reversible HER2 inhibitor lapatinib, known to promote receptor stabilization and accumulation at the plasma membrane (26),
decreased the internalization of pHrodo-T-DM1 (Fig. 3A and B). Consistently, ubiquitination of immunoprecipitated HER2 was stronger than controls upon treatment with the irreversible inhibitors neratinib or afatinib but not with the reversible inhibitors lapatinib or tucatinib in both cell lines (Fig. 3C and D). This increase in HER2 ubiquitination and internalization was recapitulated by HSP90 inhibition (Fig. 3C and D; Supplementary Fig. S4A and S4B). The same pattern of HER2 ubiquitination was reproduced also in the NCI-H2030 isogenic models (Fig. 3E). Collectively, these data suggest that irreversible inhibitors of HER2 increase polyubiquitination and subsequent internalization of the receptor.

### Table 2. T-DM1–related adverse events

<table>
<thead>
<tr>
<th>Adverse events</th>
<th>Grade 1 N (%)</th>
<th>Grade 2 N (%)</th>
<th>Grade 3 N (%)</th>
<th>Total</th>
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<tr>
<td>Elevated AST or ALT</td>
<td>28 (57)</td>
<td>3 (6)</td>
<td>—</td>
<td>31 (63)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>13 (27)</td>
<td>1 (2)</td>
<td>1 (2)</td>
<td>15 (31)</td>
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<tr>
<td>Fatigue</td>
<td>6 (12)</td>
<td>2 (4)</td>
<td>—</td>
<td>8 (16)</td>
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<tr>
<td>Nausea</td>
<td>14 (29)</td>
<td>—</td>
<td>—</td>
<td>14 (29)</td>
</tr>
<tr>
<td>Infusion reaction</td>
<td>2 (4)</td>
<td>5 (10)</td>
<td>—</td>
<td>7 (14)</td>
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<tr>
<td>Anorexia</td>
<td>3 (6)</td>
<td>2 (4)</td>
<td>—</td>
<td>5 (10)</td>
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<tr>
<td>Anemia</td>
<td>1 (1)</td>
<td>3 (6)</td>
<td>1 (2)</td>
<td>5 (10)</td>
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</table>

NOTE: Treatment-related adverse events with total frequencies of greater than 10%, according to National Cancer Institute Common Terminology Criteria for Adverse Events version 4.1 (CTCAE v4.1). There were no grade 4 or 5 adverse events.
via HSP90 dissociation in ERBB2-amplified or -mutant lung cancers. Importantly, this phenomenon seems to be prevented by reversible kinase inhibitors.

To determine whether the enhanced receptor internalization induced by cotreatment with neratinib and T-DM1 was sufficient to enhance antitumor efficacy, we treated the same lung PDXs bearing ERBB2 amplification and mutation (S310F) shown in Fig. 1J with either neratinib, T-DM1, or the combination. Although both T-DM1 and the combination of T-DM1 and neratinib induced marked tumor regression, the effect was more durable with the combination (despite negligible activity observed with neratinib monotherapy; Fig. 3F). All treatments were well tolerated by the animals (Supplementary Fig. S5).

Because the combination of T-DM1 and neratinib is not given as standard of care at progression on T-DM1, we evaluated the clinical activity of this combination in a 41-year-old patient with ERBB2-amplified breast cancer enrolled in an ongoing clinical trial (NCT01494662). At the time of study entry, the patient had relapsed after multiple lines of anti-HER2 therapy, including T-DM1 (see details in Methods). Immediately upon T-DM1 progression, neratinib was added to T-DM1 and the patient experienced a brisk partial response (38%) 6 weeks into therapy (Fig. 3G) and continued with this treatment until intracranial progression at week 18 (at that time the patient remained in confirmed partial response extracranially). This prompt response is consistent with our preclinical data that cotreatment with neratinib improved internalization and efficacy of T-DM1, and with a recently published trial showing encouraging efficacy and tolerability of T-DM1 and neratinib in metastatic breast cancer, albeit in a T-DM1–naive setting (27).

Figure 3. Pan-HER irreversible inhibitors enhance T-DM1 internalization, ubiquitination, and efficacy in both ERBB2-amplified and ERBB2-mutant lung xenografts. A Calu-3 ERBB2-amplified and phospho-T-DM1 (1 μg/mL) together with the pan-HER irreversible inhibitor neratinib (100 nmol/L), the EGFR/HER2 reversible inhibitor lapatinib (100 nmol/L), the inhibitor of endocytosis dynasore (100 μmol/L), or DMSO control for 30 minutes at 4°C and then released at 37°C and z-stack imaged every hour over 15 hours on a confocal microscope. Data are shown as number of normalized pHrodo dots per cell (trafficking index) over time. Error bars, SEM. Groups were compared with DMSO for each time point using two-way ANOVA test. **, P ≤ 0.01; ****, P ≤ 0.0001 at the indicated time point; ns, nonsignificant (n = 2 independent experiments, 80 cells analyzed in total per condition, per time point). B, LAUD-10 HER2-mutant (L755P) NSCLC cells were incubated with pHrodo-T-DM1 (10 μg/mL) together with the pan-HER irreversible inhibitor neratinib (100 nmol/L), the pan-HER reversible inhibitor lapatinib (100 nmol/L), the inhibitor of endocytosis dynasore (100 μmol/L), or DMSO control for 30 minutes at 4°C and then released at 37°C and z-stack imaged every hour over 15 hours on a confocal microscope. Data are shown as number of normalized pHrodo dots per cell (trafficking index) over time. Error bars, SEM. Groups were compared with DMSO for each time point using two-way ANOVA test. **, P ≤ 0.01; ****, P ≤ 0.0001 at the indicated time point; ns, nonsignificant (n = 2 independent experiments, 80 cells analyzed in total per condition, per time point). C, Calu-3 cells were incubated with T-DM1 (10 μg/mL) or vehicle as control, together with the pan-HER irreversible inhibitors neratinib or afatinib (100 nmol/L), the pan-HER reversible inhibitors lapatinib or tucatinib (100 nmol/L), and HSP90 inhibitor (200 nmol/L) in the presence of the proteasome inhibitor MG-132 (10 μmol/L) for 6 hours at 37°C. HER2 immunoprecipitations (IP) were performed using either T-DM1 itself or trastuzumab (added only to the protein lysates lacking T-DM1) as primary antibodies. IP or total lysate samples were evaluated by Western blot analysis. For the IP, ubiquitin and total HER2 were evaluated, showing a higher HER2 ubiquitination in the samples treated with neratinib, afatinib, or HS90 inhibitor. Ubiquitin and total HER2 were comparable among the total lysates, whereas HER2 phosphorylated on tyrosine 1248 (pHER2Y1248) demonstrated the efficacy of HER2 phosphorylation inhibition. Actin was included as loading control. D, LAUD-10 cells were incubated with T-DM1 (10 μg/mL) or IgG control, together with the irreversible HER2 inhibitors neratinib or afatinib (100 nmol/L), the reversible HER2 inhibitors lapatinib or tucatinib (100 nmol/L), and HSP90 inhibitor (100 nmol/L) in the presence of the proteasome inhibitor MG-132 (10 μmol/L) for 6 hours at 37°C. HER2 immunoprecipitations were performed using T-DM1 itself (or IgG control) as primary antibody. IP or total lysate samples were analyzed by Western blot analysis. For the IP, ubiquitin and total HER2 were evaluated. Ubiquitin and total HER2 were comparable among the total lysates, whereas HER2 phosphorylated on tyrosine 1248 (pHER2Y1248) demonstrated the efficacy of HER2 phosphorylation inhibition. Actin was included as loading control. E, Isogenic lung cancer cells NCI-H2030 ectopically expressing either WT or mutant (S310F or L755S) HER2 or transduced with an EV control were treated as in C. IP or total lysate samples were analyzed by Western blot analysis. For the IP, ubiquitin and total HER2 were evaluated. Ubiquitin and total HER2 were comparable among the total lysates, whereas HER2 phosphorylated on tyrosine 1248 (pHER2Y1248) demonstrated the efficacy of HER2 phosphorylation inhibition. Actin was included as loading control. F, In vivo efficacy study of the ERBB2S310F-mutant and -amplified lung PDX shown in Fig. 1E treated with T-DM1 (15 mg/kg, intravenously once a week), neratinib (20 mg/kg, orally every day, 5 days a week), and the combination. Measurements show average tumor volumes ± SEM, n = 7 animals per group. Comparisons between the two indicated groups for each time point were performed using two-way ANOVA test (**, P ≤ 0.01) at the indicated time point. G, CT scan of a patient with T-DM1–resistant ERBB2-amplified breast cancer who achieved an ongoing clinical trial (NCT01494662). At the time of scan acquisition, the patient was treated with T-DM1–resistant ERBB2-mediated disease. The patient achieved a partial response to T-DM1 (70% tumor shrinkage) that lasted for one year (Fig. 4B). Moreover, we observed that although lung PDXs harboring a YVMA ERBB2 mutation (different from the PDX shown in Fig. 1I) were initially sensitive to all ADC-containing therapeutically, mice receiving T-DM1 or the combination of T-DM1 and neratinib relapsed shortly after treatment discontinuation. In contrast, animals treated with T-DXd monotherapy did not show any sign of tumor regrowth up to 1 month after treatment discontinuation (Fig. 4C; Supplementary Fig. S6B). To rule out the possibility that the observed superior activity of T-DXd compared with T-DM1 monotherapy was due to faster internalization kinetics, we performed a parallel live-imaging experiment with pHrodo-T-DM1 and pHrodo-neratinib.
pHrodo-T-DXd in Calu-3 cells. As expected, we did not observe any significant difference in the internalization rate of the two ADCs (Supplementary Fig. S6C).

Collectively, these data suggest that both the combination of T-DM1 with pan-HER irreversible kinase inhibitors or switching to T-DXd result in superior activity compared with T-DM1 monotherapy (Fig. 4D).

**DISCUSSION**

The activity of T-DM1 shown here represents the first clinical trial evidence that ERBB2 amplification in lung cancers may be therapeutically targeted, which stands in contrast to two decades of disappointing efforts targeting HER2 protein expression in lung cancers (7). This study also confirmed the clinical activity of anti-HER2 ADCs in lung cancers with HER2-activating mutations, regardless of the quantity of protein expression (Supplementary Table S1). These results validate our hypothesis that receptor hyperactivation through gene amplification or mutation facilitates its ubiquitination and internalization, which may be therapeutically exploited through ADCs. Although two independently conducted clinical trials of T-DM1 targeting HER2 protein expression (IHC 2+/3+) in lung cancers did not reach expected outcomes, a signal of encouraging responses was seen in tumors with ERBB2 amplification or mutation, which is consistent with our findings (28, 29). We also observed clinical activity of T-DM1 across all activating HER2 mutation subtypes (Supplementary Table S1), in contrast to the differential activity of tyrosine kinase inhibitors across mutation subtypes seen in previous studies (11, 30, 31). This finding may be explained by the different mechanism of action of ADCs compared with tyrosine kinase inhibitors, that is, the internalization of receptor–ADC complex to deliver the cytotoxic payload rather than inhibiting oncogenic signaling. This also explains the observed

**Figure 4.** T-DXd shows increased efficacy in T-DM1–resistant tumors. 
A, In vivo efficacy study of the ERBB2<sup>S310F</sup>-mutant and -amplified lung PDX shown in Figs. 1J and 3F treated with T-DM1 (15 mg/kg, intravenously once a week) and T-DXd (10 mg/kg, intravenously once every 3 weeks). Measurements show average tumor volumes ± SEM, n = 7 animals per group. Comparisons between the two indicated groups for each time point were performed using two-way ANOVA test (****, *P* ≤ 0.0001 at the indicated time point). 
B, CT scan of the patient with ERBB2<sup>S310F</sup>-mutant and -amplified lung cancer corresponding to the PDX shown in A. Arrows point to a bone metastatic lesion at T-DM1 progression and after response to T-DXd. 
C, In vivo efficacy study of a ERBB2<sup>YVMA</sup>-mutant lung PDX treated with T-DM1 (15 mg/kg, intravenously once a week), neratinib (20 mg/kg, orally 5 days a week), T-DM1 + neratinib, and T-DXd (10 mg/kg, intravenously once every 3 weeks). Measurements show average tumor volumes ± SEM, n = 6 animals per group. Comparisons between the two indicated groups for each time point were performed using two-way ANOVA test (****, *P* ≤ 0.0001 at the indicated time point).

D, Schematic showing the two strategies proposed in this work to enhance the efficacy of anti-HER2 ADC in lung cancer: increased internalization by pan-HER irreversible inhibitors through increased ubiquitination and consequent endocytosis of the receptor–ADC complex in both ERBB2-mutant or -amplified tumors; switching anti-HER2 ADCs from T-DM1 to T-DXd and vice versa.
responses to T-DM1 in *EGFR*-mutant and *ERBB2*-amplified lung cancer. Therefore, ADCs represent a fundamentally different targeted therapy for lung cancer, as their efficacy is independent of the drug’s ability to inhibit oncogenic driver signaling.

Our laboratory and clinical data are consistent with the idea that surface-localized and cycling HER2 can serve as a carrier for HER2-specific ADCs. Therefore, strategies that augment the dynamics of internalization may increase the efficacy of these drugs. We observed that cotreatment with a pan-HER irreversible inhibitor such as neratinib enhanced internalization of HER2–ADC complexes *in vitro*, resulting in potent antitumor activity *in vivo*. Importantly, although treatment with reversible inhibitors such as lapatinib or tucatinib decreased HER2 ubiquitination, treatment with irreversible inhibitors such as neratinib or afatinib increased such ubiquitination, similar to HSP90 inhibition. A superior activity of the combination of the irreversible HER2 inhibitor poziotinib and T-DM1 compared with single agents has been recently shown in a lung cancer PDX model (32). However, the proposed mechanism of action in that report is HER2 stabilization at the membrane, which is in contrast with our findings. Moreover, it has been shown that receptor stabilization at the plasma membrane increases antibody-dependent cytotoxicity exerted by natural killer cells binding to antibodies such as trastuzumab or cetuximab (26, 33).

It has been shown that HSP90 binding regulates the stability of mature membrane-bound form of HER2. Indeed, HSP90 inhibition triggers receptor ubiquitination, destinating HER2 to proteasomal degradation (23, 34). Here, we propose that FDA-approved irreversible pan-HER inhibitors, by increasing HER2 ubiquitination, may act as HER2-specific HSP90 inhibitors. Thus, HER2 targeting by irreversible inhibitors would be used not to obtain a stronger inhibition of the kinase and the downstream signaling, but to further enhance internalization of the receptor–ADC, improving the antitumor activity of the payload. As a matter of fact, we also speculate that subtherapeutic doses (or perhaps pulsatile treatment schedules) of neratinib or afatinib may be sufficient to maximize ADC-dependent cell death/tumor shrinkage, thus minimizing the adverse effects associated with daily treatment with these agents (35).

Although combined neratinib and T-DM1 elicited an ORR of 63% with a manageable toxicity profile in a phase I trial of 19 patients with HER2-positive breast cancer previously treated with trastuzumab- and pertuzumab-based therapies (27), these responses were not compared to T-DM1 monotherapy and no data are available on the efficacy of this combination in patients with T-DM1–refractory breast cancer. Furthermore, there are no current clinical trials evaluating the clinical benefit of the combination of T-DM1 and pan-HER irreversible inhibitors in comparison with T-DM1 monotherapy in *ERBB2*-mutant or -amplified lung cancers. Similarly, it is currently unknown whether the antitumor effects of T-DXd, recently FDA-approved for metastatic breast cancer (36), would also be enhanced by the up-front combination with neratinib. The question of potential overlapping gastrointestinal toxicity of the two agents may only be addressed through clinical trial investigation.

The superior efficacy of T-DXd compared with T-DM1 observed in our models is unlikely to be the consequence of differential ADC internalization. Rather, we posit that genetic, epigenetic, or transcriptional mechanisms that render tumor cells more refractory to microtubule-directed chemotherapy (37, 38) may be at play in this context. The antitumor activity of T-DXd in tumors that are or become resistant to T-DM1 may be the result of a combination of variables, including the higher drug-to-antibody ratio compared with T-DM1, diversity between stability and cleavability of the respective linkers, a cytotoxic payload with a different mechanism of action, and the higher cell permeability of the payload resulting in bystander cytotoxicity in the neighboring cells (39). The identification of biomarkers of response to the available ADCs (or their payloads) will be critical for a better patient stratification in future trials.

Despite the earlier setbacks, several clinical development strategies targeting HER2 in lung cancers are now under way. T-DXd is currently being evaluated in phase I/II clinical trials as a single agent (NCT03505710) or in combination with immune checkpoint inhibitor pembrolizumab (NCT04042701). Trastuzumab duocarmazine is another anti-HER2 ADC which has demonstrated activity in patients with T-DM1–resistant or low HER2–expressing breast cancers (40). Other anti-HER2 ADCs in early-phase development include ZW-49 (NCT03821233), DHES0815A (NCT03451162), RC48-ADC (NCT03500380), and A166 (NCT03602079). Novel irreversible pan-HER tyrosine kinase inhibitors such as pyro tinib and poziotinib have also shown promising activity in early-phase clinical trials targeting HER2-mutant lung cancers (32, 41). As the development of brain metastases is common in HER2-mutant lung cancers, affecting 47% of patients, central nervous system activity should be further evaluated in future trials as it is in breast cancer trials (42, 43). As these trials of HER2-targeted agents bring renewed hope, our findings provide clinical evidence and mechanistic insights to guide the development of ADCs and their combinations for patients with lung cancers and other solid tumors.

In summary, ADC-based therapies are a promising new treatment for patients with *ERBB2*-amplified or -mutant lung cancers. Concurrent treatment with irreversible pan-HER kinase inhibitors or ADC therapy switching can improve the efficacy of these agents. Our findings can potentially be extended to other HER2-activated tumor types for which pharmacologic HER2 signaling blockade is insufficient to elicit strong and durable responses, and may help guide future clinical trials and expand the field of ADCs as cancer therapy.

**METHODS**

**Ethical Compliance**

We declare compliance with all relevant ethical regulations.

**Patients**

A cohort of 49 patients with lung adenocarcinomas carrying either amplification of or mutations in *ERBB2* was enrolled in a phase II basket trial at MSK #15-335 (NCT020267829) to assess the clinical activity of ado-trastuzumab emtansine (T-DM1). The primary objective was the determination of ORR according to RECIST v1.1 or modified PERCIST criteria as assessed by investigator. Secondary objectives included assessment of PFS and toxicity according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.1 (NCI CTCAE v4.1). The data cutoff for this analysis was August 1, 2019. All
patients received T-DM1 at 3.6 mg/kg by intravenous infusion every 21 days until disease progression or unacceptable toxicity. Physical examination and safety assessments were performed every 3 weeks. Tumor assessments using contrast enhanced CT chest abdomen pelvis and/or PET were performed at baseline, week 6, week 12, then every 12 weeks thereafter until disease progression. One patient had no RECIST or PERCIST measurable disease but was evaluable for progression-free survival. Therefore, the waterfall plot included 48 patients. The 95% exact CI for ORR was calculated using the Clopper-Pearson method. PFS time was estimated by the Kaplan–Meier method. ERBB2 mutation and amplification status was evaluated using MSK-IMPACT (see targeted tumor sequencing section below), another NGS assay in a CLIA-certified laboratory, and/or FISH. FISH was performed using FDA-approved probe sets (PathVysion, Abbott, and ERBB2 IQFISH pharmDx, Dako).

HER2 protein was assessed by IHC using the 4B5 antibody (Ventana).

All patients provided written informed consent for genomic sequencing of tumor DNA, and review of medical records for detailed demographic, pathologic, and clinical data and for publication of this information as part of an institutional review board (IRB)-approved investigator sponsored trial (NCT02675829). Research protocols for tumor collection and analysis were approved by the IRB of Memorial Sloan Kettering Cancer Center (MSK).

The patient shown in Fig. 3F has provided written informed consent to DF/HCC protocol #09-204, which is an IRB-approved, prospective cohort study enrolling patients with metastatic breast cancer seen for at least one visit to Dana-Farber Cancer Institute (Boston, MA). The consent includes permission to publish deidentified data. In addition, the patient consented to DF/HCC protocol #11-344, a prospective clinical trial testing neratinib-based therapies for patients with breast cancer brain metastases (NCT01494662), and received the combination of neratinib + T-DM1 under this protocol. The investigator-sponsored trial’s principal investigator (Dr. Rachel Freedman) has provided permission to report on the extracranial response only for this single trial patient ahead of reporting of the primary trial endpoint (central nervous system objective response rate). Before being enrolled in this clinical trial, the patient was treated with doxorubicin–cyclophosphamide followed by pacitaxel–trastuzumab–pertuzumab, achieving a pathologic complete response at surgery. The patient then completed adjuvant trastuzumab treatment and started docetaxel–trastuzumab–pertuzumab, when metastatic relapse (bilateral pulmonary nodules and mediastinal/hilar/supraclavicular adenopathy) occurred 27 months after completion of adjuvant trastuzumab treatment. The patient achieved a clinical response and was transitioned to trastuzumab–perixezol–pertuzumab maintenance. Seven months after the initial diagnosis of metastatic disease, symptomatic brain metastases were diagnosed, and treated with surgery followed by whole-brain radiotherapy. Trastuzumab and pertuzumab were continued for an additional 2 months; however, the patient progressed with pericardial effusion and pulmonary metastases, and T-DM1 monotherapy was started. Tumor burden stabilized on T-DM1 and was evaluable for progression-free survival. The patient was then treated with T-DXd on a phase 1 clinical trial (NCT02564900) and achieved a partial response with 70% tumor shrinkage on RECIST v1.1 with clinical benefit that lasted 1 year.

**Cell Lines**

MCF10A isogenic cell lines were purchased from ATCC (CRL-10317) and were grown in DMEM/F12 (Invitrogen #11330-032) supplemented with horse serum 5% (Invitrogen #10050-122), EGF 20 ng/mL (PeproTech #10-100-15), hydrocortisone 0.5 mg/mL (Sigma #H-8882), cholera toxin 100 ng/mL (Sigma #C-8052), insulin 10 μg/mL (Sigma #I-8882), penicillin/streptomycin 1% under standard conditions.

NCI-H2030 were purchased from ATCC (CRL-5914) and were grown in RPMI 1640 (Thermo Fisher Scientific, #11875093) supplemented with 10% heat-inactivated FBS (Thermo Fisher Scientific, #16140071), penicillin/streptomycin 1% under standard conditions.

Patient-derived LUAD-10 cells were established in our laboratory from a PDX engrafted with the pellet isolated from the pleural effusion of a patient with HER2<sup>Leu755Pro</sup>-mutant lung cancer from a phase II basket trial at MSK #15-335 (NCT02675829), after T-DM1 progression. Cells were plated on collagen-coated Petri dishes and cultured in DMEM/F12 supplemented with 10% FBS, penicillin/streptomycin 1% under standard conditions.

**Lentiviral Infections**

MCF10A and NCI-H2030 isogenic cell lines stably expressing ERBB2 WT, or ERBB2 mutants were obtained by lentiviral infection as follows. Briefly, 7 × 10<sup>9</sup> 293T cells were seeded into 10-cm dishes. Cells were transfected with 1.2 μg pMD2.G, 2.4 μg pCMV-dR8.2, and 3.6 μg pLX302-EV, pLX302-ERBB2 WT, pLX302-ERBB2 S310F, or pLX302-ERBB2 L755S using jetPRIME (Polyplus-transfection) following the manufacturer’s instructions. Medium was refreshed 6 hours post-transfection. The supernatant was collected 72 hours and filtered with 0.45-μm filters.

MCF10A and NCI-H2030 cells (3 x 10<sup>4</sup>) were seeded into 6-well plates and transduced with different dilutions of freshly collected lentiviruses with 8 μg/mL Polybrene for 24 hours. Cells were then transferred into 10-cm dishes and selected with 2 μg/mL (MCF10A) or 5 μg/mL (NCI-H2030) of puromycin for 2 days. Cells stably expressing comparable levels of WT or mutant HER2 were chosen for subsequent studies.

**pHrodo-ADC Assay**

pHrodo-TDM1 and pHrodo-T-DXd were generated using pHrodo iFL Red Microscale Protein Labelling Kit (Invitrogen #P36014) according to manufacturer’s instructions. Briefly, a reaction solution containing 100 μL of the ADC (1 mg/mL), 10 μL of sodium bicarbonate...
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(1 mol/L), and 3.3 μl of pHrodo iFl Red STP esterase-reactive dye (2 nmol/L in DMSO) was incubated for 30 minutes at room temperature. pHrodo-ADCs were purified by centrifugation using a purification resin, aliquoted, and stored at 20°C.

For internalization assays, 4 × 10⁵ cells were seeded on 8-well chamber slides (Nunc Lab-Tek II Chamber Slide, Thermo Fisher Scientific #154534). The day after, cells were incubated with serum-free medium containing Hoechst (Thermo Fisher Scientific #62249) for live-cell fluorescent staining of DNA, and pHrodo-ADC (1 μg/mL, unless stated differently in the figure legend) for 30 minutes at 4°C and then released at 37°C in the heated live chamber of the Zeiss LSM880 confocal microscope. Positions were decided using brightfield (at least 4 positions per sample) and 2 μm-thick z-stack images of brightfield and red fluorescence were taken every hour over 16 hours. Images were then analyzed using Fiji software and results were displayed as average number of cytoplasmic red fluorescent dots per cell (trafficking index).

Where indicated, cells were incubated with serum-free medium containing Hoechst stain, pHrodo-ADC, and neratinib (200 nmol/L, unless stated differently in the figure legend) or lapatinib (100 nmol/L, unless stated differently in the figure legend) or HSP90 inhibitor (200 nmol/L) or DMSO as control.

Western Blots

Total protein lysates (20 μg) were extracted using RIPA buffer and separated on SDS-PAGE gels (NuPAGE 4–12 Bis-Tris Protein Gels, Invitrogen) according to standard methods.

Membranes were probed using the following antibodies: anti-total HER2 Rabbit mAb (29D8, Cell Signaling Technology #2165), anti-total HER2 Mouse mAb (CB11, Thermo Fisher Scientific #MA1-35720), anti-phospho-HER2 (Tyr1248, Cell Signaling Technology #2247), anti-Cleaved PARP Asp214 human specific (Cell Signaling Technology #9541), anti-phospho-tyrosine (Cell Signaling Technology #9854), anti-β-Actin 13E5 (Cell Signaling Technology #4970), and ubiquitin (P4D1, Cell Signaling Technology #39365).

HER2 Immunoprecipitation

For immunoprecipitation (IP) assays, cells were incubated in 5% FBS medium in the presence of the proteasome inhibitor MG-132 (10 μmol/L), together with 10 μg/mL of T-DM1 (or IgG control, Cell Signaling Technology #2729) and 100 nmol/L of tyrosine kinase inhibitors or HSP90 inhibitor at 37°C for 6 hours (except for LUAD-10, which were incubated for 3 hours). Cells were then washed in cold PBS and lysed using NP-40 buffer (150 mmol/L NaCl, 10 mmol/L Tris pH 8, 1% NP-40, 10% glycerol). Twenty micrograms of proteins were used as total lysates. Protein lysates (350 μg for Calu-3 and NCI-H2030, 1500 μg for LUAD-10) were incubated rotating at 4°C for 4 hours in the presence of 10 μg/mL trastuzumab, which was added only to samples lacking T-DM1 or IgG control. Agarose Protein A/G beads (Santa Cruz Biotechnology #sc-2003) were then added and samples were incubated rotating at 4°C for 1 hour. Beads were centrifuged at 3,000 rpm for 1 minute and supernatant was removed. Similarly, beads were washed twice using NP-40 buffer and once using nuclease-free sterilized water. Finally, immunoprecipitates were eluted using 1x sample buffer (NuPAGE LDS Sample Buffer, Thermo Fisher Scientific #NP0008 and NuPage Sample Reducing Agent, Thermo Fisher Scientific #NP0009) at 95°C for 5 minutes.

In Vivo Studies

PDx were generated as follows: 6-week-old NOD scid gamma female mice were implanted subcutaneously with specimens freshly collected from patients at MSK under an MSK-approved IRB biospecimen protocol #06-107. Tumors developed within 2 to 4 months and were expanded into additional animal mice by serial transplantation.

At this point, PDxs were subjected to high-coverage NGS with the MSK-IMPACT assay. For efficacy studies, treatment was started when tumor volumes reached approximately 100 mm³. Xenografts were randomized and dosed with T-DM1 (15 mg/kg, intravenously once a week), neratinib (20 mg/kg, orally 5 days a week), a combination of the two agents, T-DXd (10 mg/kg, intravenously once every 3 weeks), or vehicle as control (saline, orally 5 days a week). Mice were observed daily throughout the treatment period for signs of toxicity. Tumors were measured twice weekly using calipers, and tumor volume was calculated using the formula length × width² × 0.52. Body weight was also assessed twice weekly. At the end of each treatment, animals were sacrificed, and tumors were collected for biochemistry and histology analysis. Mice were cared for in accordance with guidelines approved by the MSK Institutional Animal Care and Use Committee and Research Animal Resource Center. Six to 7 mice per group were included in each experiment.

Statistical Analysis

Statistical analyses were conducted using GraphPad Prism 8 (GraphPad Software Inc.). Two-way ANOVA test was used to evaluate significant differences in tumor volumes in in vivo efficacy studies. Data are presented as mean ± SEM. *, P ≤ 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001. Median PFS and 95% CI were calculated using the Kaplan–Meier method. Association of EBBR2 copy number with response status was evaluated using the Wilcoxon rank-sum test. Spearman correlation was used for correlating copy-number assessments by FISH, IHC, and NGS.

Data Availability

All genomic results and associated clinical data for all patients in this study are publicly available in the cBioPortal for Cancer Genomics at the following URL: http://cbiportal.org/msk-impact. All other relevant data are included in the article and/or Extended Data files.

Disclosure of Potential Conflicts of Interest

B.T. Li is a consultant/advisory board member at Roche/Genentech, Bioscope International, Thermo Fisher Scientific, Mersana Therapeutics, Hengrui Therapeutics, and Guardant Health, reports receiving commercial research grants from Roche/Genentech, Daiichi Sankyo, Lilly, Hengrui Therapeutics, Illumina, Guardant Health, BioMed Valley Discoveries, AstraZeneca, GRAIL, MORE Health, and Agen, and has ownership interest in two pending institutional patents at Memorial Sloan Kettering Cancer Center (US62/685,057, US62/514,661). S. Misale is a consultant at Boehringer-Ingelheim. A.S. Lalani is SVP, Clinical Science, at Puma Biotechnology. N.U. Lin is an advisory board member at Puma, Seattle Genetics, and Daiichi Sankyo and reports receiving commercial research grants from Genentech, Merck, Pfizer, and Seattle Genetics. D.B. Solit is an advisory board member at Pfizer, Loxo Oncology, Lilly Oncology, Vividion Therapeutics, Illumina, and QED Therapeutics. M.F. Berger is a consultant at Roche. T. Ng reports receiving commercial research grants from Daiichi Sankyo and AstraZeneca. M. Offin has received speakers bureau honoraria from PharmaMar, Novartis, and Targeted Oncology. J.M. Isbell has ownership interest (including patents) in LumaCyte, LLC, and is a consultant/advisory board member for Roche Genentech. D.R. Jones is senior medical advisor at Diffusion Pharmaceuticals, and a consultant at Merck and AstraZeneca. H.A. Yu is a consultant at AstraZeneca and Daiichi and reports receiving commercial research grants from AstraZeneca, Pfizer, Daiichi, Novartis, and Lilly. S. Thyparambil is a senior director, R&D, at mProbe. A. Bhalkikar is an associate scientist at mProbe, Inc. F. Cecchi is an associate director, proteomics,
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REFERENCES


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