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ORIGINAL ARTICLE

Ado-trastuzumab emtansine (T-DM1) in patients with HER2-amplified tumors excluding breast and gastric/gastroesophageal junction (GEJ) adenocarcinomas: results from the NCI-MATCH trial (EAY131) subprotocol Q

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Background: The National Cancer Institute—Molecular Analysis for Therapy Choice (NCI-MATCH) is a national precision medicine study incorporating centralized genomic testing to direct refractory cancer patients to molecularly targeted treatment subprotocols. This treatment subprotocol was designed to screen for potential signals of efficacy of ado-trastuzumab emtansine (T-DM1) in *HER2*-amplified histologies other than breast and gastroesophageal tumors.

Methods: Eligible patients had *HER2* amplification at a copy number (CN) >7 based on targeted next-generation sequencing (NGS) with a custom Oncomine AmpliSeq™ (ThermoFisher Scientific) panel. Patients with prior trastuzumab, pertuzumab or T-DM1 treatment were excluded. Patients received T-DM1 at 3.6 mg/kg i.v. every 3 weeks until toxicity or disease progression. Tumor assessments occurred every three cycles. The primary end point was centrally assessed objective response rate (ORR). Exploratory end points included correlating response with *HER2* CN by NGS. The impact of co-occurring genomic alterations and PTEN loss by immunohistochemistry were also assessed.

Results: Thirty-eight patients were enrolled and 36 included in efficacy analysis. Median prior therapies in the metastatic setting was 3 (range 0–9; unknown in one patient). Median *HER2* CN was 17 (range 7–139). Partial responses were observed in two (5.6%) patients: one mucoepidermoid carcinoma of parotid gland and one parotid gland squamous cell cancer. Seventeen patients (47%) had stable disease including 8/10 (80%) with ovarian and uterine carcinomas, with median duration of 4.6 months. The 6-month progression-free survival rate was 23.6% [90% confidence interval 14.2% to 39.2%]. Common toxicities included fatigue, anemia, fever and thrombocytopenia with no new safety signals. There was a trend for tumor shrinkage with higher levels of gene CN as determined by the NGS assay.

Conclusion: T-DM1 was well tolerated. While this subprotocol did not meet the primary end point for ORR in this heavily pretreated diverse patient population, clinical activity was seen in salivary gland tumors warranting further study in this tumor type in dedicated trials.

Key words: *HER2*-amplified, T-DM1, NCI-MATCH, NGS

Introduction

The 'National Cancer Institute - Molecular Analysis for Therapy Choice' (NCI-MATCH) trial is a national signal-finding precision medicine study that incorporates genomic testing to direct refractory cancer patients to molecularly targeted treatments. The NCI-MATCH is a single protocol (EAY131) which incorporates nearly 40 phase II treatment subprotocols (Clinical Trials.gov, NCT02465060).

To date, the vast majority of precision medicine trials have relied upon next-generation sequencing (NGS) assays of archival tumor tissue to determine the genomic profile of a tumor. A unique feature of the NCI-MATCH trial is that it incorporated a centralized validated assay that analyzed freshly acquired samples collected at over 1100 sites and used four Clinical Laboratory Improvement Amendments (CLIA)-accredited NCI-MATCH network laboratories that were harmonized for assay concordance. Targeted NGS was performed using the Ion Torrent Oncomine AmpliSeq^{TM} panel of 143 genes and was supplemented with Phosphatase and tensin homolog (PTEN), MutS homolog (MSH) and MutL homolog (MLH) immunohistochemistry (IHC). All assays were performed in CLIA laboratories under an Investigational Device Exemption (IDE).

One of the genes on the panel was the *ERBB2* (*HER2)* gene that encodes a member of the *ERBB* family of receptor tyrosine kinases and is a key proto-oncogene in solid tumors [1, 2]. *HER2* amplification is a critical oncogenic driver event found in approximately 15% to 20% of breast and gastroesophageal cancers [3, 4]. To that end, successful application of *HER2*-directed therapies has improved the overall survival (OS) of patients with early and advanced *HER2* positive (overexpressed and/or amplified) breast cancer [5–9]. In gastric cancer, the addition of trastuzumab to chemotherapy in the metastatic setting led to an improvement in OS and established a new standard of care for these patients [4, 10, 11]. *HER2* amplifications also occur in a variety of other solid tumors including lung, bladder, endometrial cancers for which no *HER2*-directed therapy is currently approved [12–20]. In one large cohort, the overall frequency of *HER2* amplification was 2% across multiple tumors (excluding breast, gastric and gastroesophageal cancers) with considerable variation by individual tumor type [21, 22] (Figure 1). Furthermore, *HER2* amplification may also be implicated in chemoresistance and overall poor survival in lung, bladder, cervical, endometrial and ovarian cancers, which underscores the unmet need for effective *HER2* directed therapies for these patients [23–26].

Ado-trastuzumab emtansine or T-DM1 is an antibody drug conjugate (ADC) linking trastuzumab coupled via a noncleavable thioether linker to 3–4 molecules of the maytansine derivative DM1. It is currently approved for the treatment of *HER2*-amplified and/overexpressed metastatic breast cancer based on the progression-free and OS benefit in the second- and third-line metastatic settings, respectively [6, 27]. The objective response rate (ORR) in *HER2*-amplified and/overexpressed gastric and metastatic breast cancer in the second-line setting was 21% and 44%, respectively [6]. This treatment subprotocol (EAY131- Q) of the NCI-MATCH protocol is investigating the activity of T-DM1 in *HER2*-amplified nonbreast and nongastric or gastroesophageal junction solid tumors.

Methods

Patient eligibility and assays

The NCI-MATCH master protocol included patients with solid tumors, lymphomas and multiple myeloma whose disease had progressed following at least one line of standard systemic therapy or for whom no standard therapy was available. Participation occurred in two phases: screening phase and treatment phase. As part of the screening phase, consented patients underwent a fresh tumor biopsy. Analysis was then performed using a centralized, customized Thermo Fisher Oncomine AmpliSeq^{TM} NGS panel and PTEN immunohistochemistry for expression of PTEN under an IDE submitted to the investigational new drug application held by NCI [28, 29]. Patients whose tumors contained amplification of *ERRB2* of more than copy number (CN) 7 (validated limit of detection) by NGS and for whom all other eligibility requirements from the master protocol were met (supplementary data, available at *Annals of Oncology* online) were offered participation in this subprotocol.

For this treatment subprotocol, key eligibility requirements included any solid tumor except breast or gastric/gastroesophageal junction cancer and presence of measurable disease, defined by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 criteria [30]. Additional eligibility requirements included Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, absolute neutrophil count of $\geq$1500/μl, platelet count $\geq$100,000/μl, hemoglobin concentration $\geq$9 g/dl, as well as adequate kidney and liver function. Patients with prior HER2 therapies (approved or investigational) and a left ventricular ejection fraction of <50% were excluded (supplementary data, available at *Annals of Oncology* online).

The study was approved by the NCI Central Institutional Review Board and is listed in clinicaltrials.gov (NCT02465060).

Treatment and evaluation

Patients were treated with the standard intravenous dosing of T- DM1, that is 3.6 mg/kg every 3 weeks for a 21-day cycle, until toxicity or progression. Imaging was performed every three cycles for the first 33 cycles and every four cycles thereafter.

Statistical methodology and end point analysis

The primary end point was ORR, defined as a complete or partial response, consistent with RECIST version 1.1 criteria for solid tumors. Allowing for 10% ineligibility rate, the accrual goal was 35 patients. However, additional patients were allowed to enroll for an additional 6 months or until the activation of another subprotocol for this population, provided outcome data were unavailable on at least 31 patients. The proposed design had the operating characteristics of at least 92% power

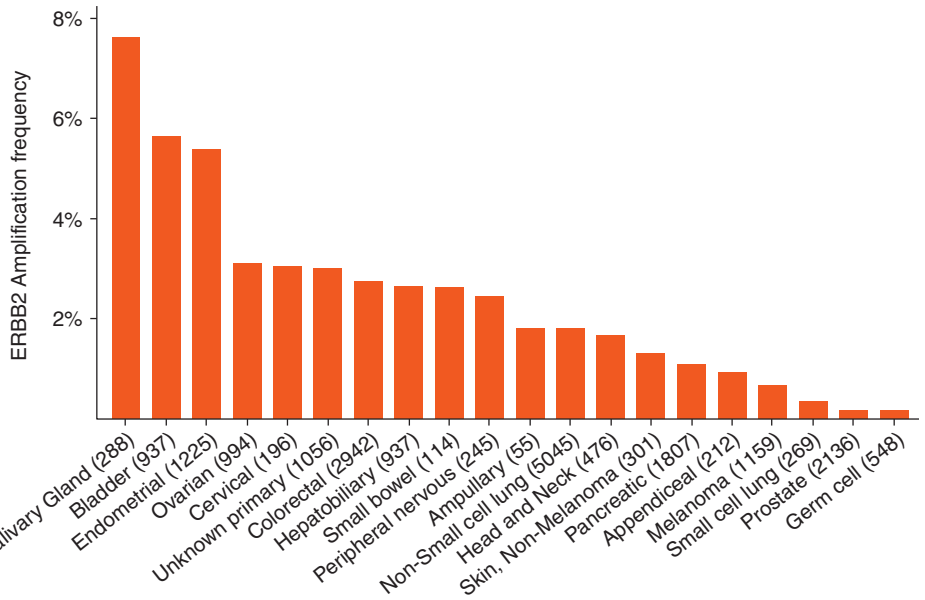

Figure 1. *ERBB2* amplification frequency: all tumors excluding breast and gastric/gastroesophageal junction tumors. This plot includes solid tumors (467/28 106 samples and 442/25 637 patients) with a minimum of 50 sequenced tumors and *ERBB2* amplification > 0% using MSK-IMPACT assay. The numbers in the parentheses are the total number of sequenced tumors. (cBioPortal.org).

to distinguish an ORR of 25% from a null of 5% with one-sided Type 1 error of 1.8%. T-DM1 would be declared promising and worthy of further study if ≥5/31 (16%) patients achieved ORR. Secondary end points included progression-free survival (PFS) at 6 months, PFS and OS. Exploratory end points included correlating response with *HER2* CN by NGS. The impact of co-occurring genomic alterations and PTEN loss by immunohistochemistry were also assessed.

Results

Thirty-eight patients with a tumor *ERBB2* CN of >7 were assigned and enrolled on this subprotocol arm. These patients received at least one dose of treatment and were included in the safety analysis. Two patients did not meet other treatment eligibility criteria by central review leaving a final cohort of 36 eligible patients that were included in the efficacy analysis (Figure 2). The first patient was enrolled for treatment on 19 November 2015 and the last patient was enrolled on March 20, 2017. Baseline characteristics are listed in Table 1. Median age was 64 (range 39–80 years). Median number of prior treatment regimens in the metastatic setting was 3 (range 0–9; unknown for one patient). Median *ERBB2* CN was 17 (range 7–139). Twenty-two different tumor types enrolled to this subprotocol (Table 1).

Efficacy

As of the data lock for analysis on 9 March 2019, the ORR was 2/36 (5.6%) with 90% confidence interval (90% CI, 1.0% to 16.5%). In addition, 17 patients had stable disease as best response (SD, 47.2%), while 13 patients had progressive disease (36.1%). Four patients were not evaluable for response due to death before first assessment scan or timing of assessments. The 6-month PFS rate was 23.6% (90% CI 14.2% to 39.2%). Median treatment duration was four cycles (range 1–37). The median PFS was 3.1 months (90% CI 2.1–4.4 months) (Figure 3C). Thirty deaths have been reported, with a median OS of 8.4 (90% CI 4.7–11.8) months.

Some reduction in tumor size was seen in 17 patients (Figure 3). Of the seven patients with more than 30% reduction in the sum of diameters of measurable tumors, two patients with salivary gland cancers (one patient each with mucoepidermoid carcinoma of the parotid gland and squamous cell carcinoma of the parotid gland) were confirmed to be partial responders (Figure 3A). Of note, the patient with parotid gland squamous cell carcinoma who achieved a PR remains on therapy at 23.7 months and the duration of treatment response for the patient with mucoepidermoid carcinoma of the parotid gland was 9 months. A subset of patients achieved SD for >6 months including patients with gynecological and colorectal cancers (Figure 3B).

Safety

All patients who received at least one dose of the study drug were included in the safety analysis. No new safety signals were seen with T-DM1 with expected and known side-effects of fatigue, nausea, elevated liver enzymes and thrombocytopenia which were predominantly grade 1 and 2 (Common Terminology Criteria for Adverse Events (CTCAE) v5.0) (Table 2). There were no deaths related to T-DM1.

Correlative analyses

There was a trend for tumor shrinkage with higher levels of gene CN as determined by the NGS assay (Figure 4).The CN for the two partial responders was 139 and 21, respectively. In a linear model, there was an estimated tumor shrinkage of 24.5% (95% CI 42.6% to 6.4%) with each doubling of CN ($P = 0.01$).

The most common co-occurring mutation was a missense *TP53* mutation seen in 89% of the patients which is higher than expected. For instance, in HER2-enriched primary breast cancer,

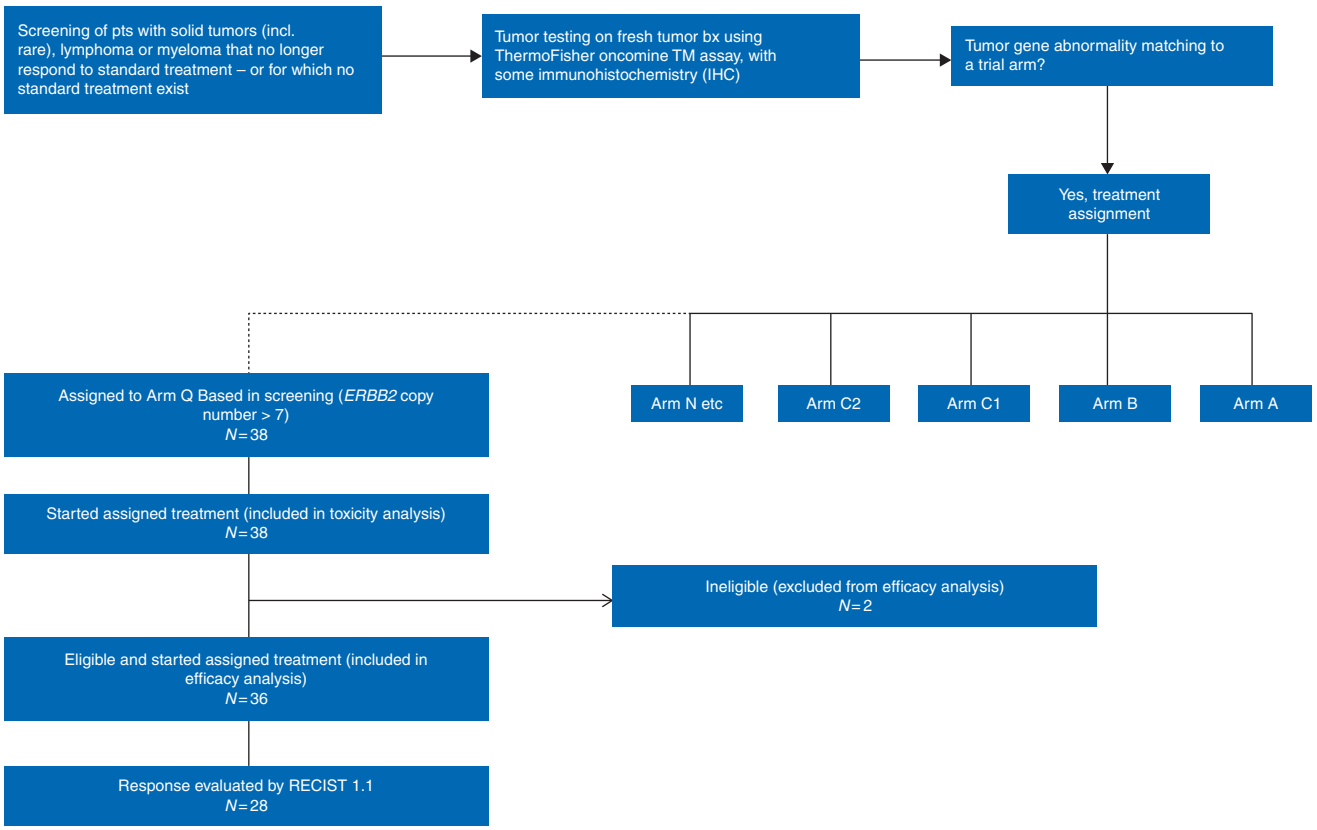

Figure 2. Consort diagram.

the prevalence of *TP53* mutation was 70% [31] and *TP53* mutations were more than fourfold enriched in MSK-IMPACT compared with TCGA (29% versus 7%) [22]. This might suggest that our cohort enriched for patients with more clinically aggressive disease [22]. The presence and nature of other co-occurring mutations in a given disease type in this cohort was as expected. For example, 73% (8/11) of colorectal patients had a frameshift/nonsense *APC* mutation without any concurrent *RAS* mutation. Interestingly, one patient with rectal adenocarcinoma had a co-occurring missense *ERBB2* V777L mutation and achieved tumor shrinkage (Figure 5). Of note, responses with T-DM1 have been reported in *HER2* mutant lung cancer [32]. Only seven patients had a co-occurring *PIK3CA* mutation. Overall, the presence of any specific co-occurring genomic alteration did not correlate with outcome, but this analysis was limited by the overall cohort size and small number of responders.

Paired pre- and posttreatment biopsy in a patient with colon adenocarcinoma, who had an unconfirmed PR and progressed after seven months, showed no differences in the genetic alterations captured by our screening test. Interestingly, sequencing of a posttreatment biopsy from the patient with mucoepidermoid carcinoma of parotid gland, with a pretreatment *HER2* CN of 21.06, revealed loss of the *HER2* amplification but persistence of a frameshift *TP53* mutation.

Discussion

Molecular profiling and genomically guided therapies have been implemented in routine clinical practice for many cancers, e.g. non-small-cell lung cancer, melanoma, gastrointestinal stromal tumors, basal cell carcinomas, and others [33–35]. However, many tumor types have a low incidence of targetable molecular alterations (usually <5%) which makes tumor-directed biomarker selected drug development studies challenging. One approach has been to utilize 'basket trials' that determine eligibility by the presence of a biomarker rather than a tumor type [36–38]. The NCI-MATCH trial is unique in that it systematically leveraged a centralized assay to explore many treatment arms in parallel from patients at over 1000 clinical sites under a master protocol. This report concerns the subprotocol evaluating T-DM1, an ADC for *HER2*-amplified nonbreast and nongastric/gastroesophageal junction cancers.

The ORR (5.6%) in this subprotocol did not meet the prespecified threshold for ORR (defined as ≥5 responses in 31 patients or 16%). While this is disappointing, there are several possible reasons for this apparent low response rate. First, this treatment subprotocol enrolled heavily pretreated patients with multiple unique histologies and a clinically aggressive phenotype (as demonstrated by enrichment for *TP53* mutations in 89% of patients; Figure 5). It is possible that response may be histology dependent. Moreover, response evaluation using RECIST v1.1 was available in only 77% (28/36) patients. Despite that, confirmed and durable partial responses were seen in two out of the three salivary gland tumors. Notably, complete and partial responses have been reported in 90% (9/10 patients) salivary gland cancers in another phase II multihistology basket trial of T-DM1 in *HER2*-amplified solid tumors [39]. This warrants further study of T-DM1 in salivary gland tumors in larger dedicated trials. Additionally, there

were 4 unconfirmed partial responses and 13 others that achieved disease stabilization (SD: 17/36 [47%]) including a subset of patients with colorectal and ovarian cancers that had SD of >6 months, which is in line with what has been reported with other anti-*HER2* therapies and T-DM1, respectively, in other multihistology basket trials [40, 41].

The evaluation of HER2 status by HER2 protein overexpression by immunohistochemistry (IHC 3+) is established as an accepted alternative to assessment of gene amplification by FISH [2, 4, 5]. However, in breast cancer, the criteria for *HER2* positivity as defined using the American Society of Clinical Oncology/College of American Pathologists clinical practice guidelines based on IHC and/or FISH, are slightly different than the criteria for *HER2* positivity in gastroesophageal adenocarcinomas [42, 43]. Hence, it is certainly possible that HER2 positivity using IHC and/or FISH could each apply to only some tumor types. *ERBB2* amplification can also be reliably determined by NGS. Notably, amplification calls using the hybrid capture-based MSK-IMPACT NGS assay had an overall concordance of 98% when compared with IHC and/or FISH [44, 45]. Similarly, the sensitivity of *HER2* amplifications calls using the NCI MATCH assay was > 92% based on orthogonally validated tests including FISH. However, screening the target using NGS does not account for spatial heterogeneity. It is well known that the sensitivity to treatment is higher when *HER2* is highly expressed or amplified [46, 47]. To that end, a trend for tumor shrinkage with T-DM1 was associated with higher levels of gene CN as determined by the NCI-MATCH assay in this subprotocol. Furthermore, a patient with mucoepidermoid tumor of the parotid gland, with an *HER2* CN of 21 at baseline, who remained on therapy for 9 months before progression, had a post progression biopsy that showed no *HER2* amplification. It is possible that loss of *HER2* amplification is related to spatial heterogeneity, i.e. biopsy of a *HER2* negative subclone, but could also be explained as a resistance mechanism to T-DM1 therapy. Loss of *HER2* amplification as a mechanism of resistance to *HER2*-directed therapy has been previously demonstrated in breast and gastric cancer [48–50]. Alternative attractive strategies such as cell-free circulating tumor DNA (ctDNA)-based NGS can provide a real-time profile of a tumor's genomic landscape in a dynamic (serial) fashion, attempting at the same time to recapitulate tumor heterogeneity and treatment response and resistance mechanisms [47, 51].

Lastly, newer ADCs with more potent payloads and bystander effects might further improve responses in *HER2* expressing tumors [52]. For example, DS-8201a, another *HER2*-targeting ADC, with a novel topoisomerase I inhibitor and high drug-to-antibody ratio of 7 to 8 : 1 which is higher than that of T-DM1 (3.5), has shown broader antitumor activity preclinically and activity in HER2 overexpressing solid tumors, and is also being evaluated in tumors with low HER2 expression [40].

In conclusion, this treatment subprotocol of *HER2*-amplified nonbreast and nongastric/gastroesophageal junction tumors treated with ado-trastuzumab emtansine did not meet the predefined threshold of ORR in this heavily pretreated tumor agnostic cohort, despite the established activity and approval of this therapy for *HER2*-amplified metastatic breast cancer. However, the activity in *HER2*-amplified salivary gland tumors, seen in this subprotocol and other trials, warrants further investigation of the efficacy of treatment with this agent or other novel antibody drug conjugates for these tumors in larger trials.

Table 1. Patient demographics

	Total (*n* = 36)
Female	23 (64%)
Median age	64 (39–80)
Race	
White	26 (72%)
Black	5 (14%)
Asian	2 (6%)
Native American	1 (3%)
Unknown/not reported	2 (6%)
ECOG Performance Status 0	12 (32%)
Prior lines of therapy in the metastatic setting	
0	1 (3%)
1	5 (14%)
2	11 (31%)
>3	18 (51%)
Median *ERBB2* copy number	17 (7–139)
Tumor type	*n* (%)
Lower gastrointestinal malignancies	11 (31)
Colon adenocarcinoma	7
Rectal adenocarcinoma	4
Gynecological (GYN) malignancies	14 (39)
Serous adenocarcinoma of ovary (one with mucin vacuoles)	3
Serous adenocarcinoma of fallopian tube	1
Mixed serous and endometrioid endometrial adenocarcinoma	2
Papillary serous endometrial adenocarcinoma	1
Malignant mixed Mullerian tumor/ carcinosarcoma	1
Mucinous adenocarcinoma of cervix/adenoma malignum	1
GYN primary site not definable	
Serous Adenocarcinoma	3
Papillary serous adenocarcinoma	1
Clear cell adenocarcinoma	1
Lung carcinoma	4 (11)
Adenocarcinoma of lung	1
Squamous cell carcinoma of lung	1
Adenosquamous carcinoma of lung	1
Squamous cell carcinoma of bronchus	1
Biliary adenocarcinoma	3 (8)
Extrahepatic cholangiocarcinoma	1
Intrahepatic cholangiocarcinoma	1
Gallbladder adenocarcinoma	1
Salivary gland tumors	3 (8)
Mucoepidermoid carcinoma of Parotid gland	2
Squamous Cell Carcinoma of Parotid Gland	1
Extramammary Paget's of scrotum	1 (3)

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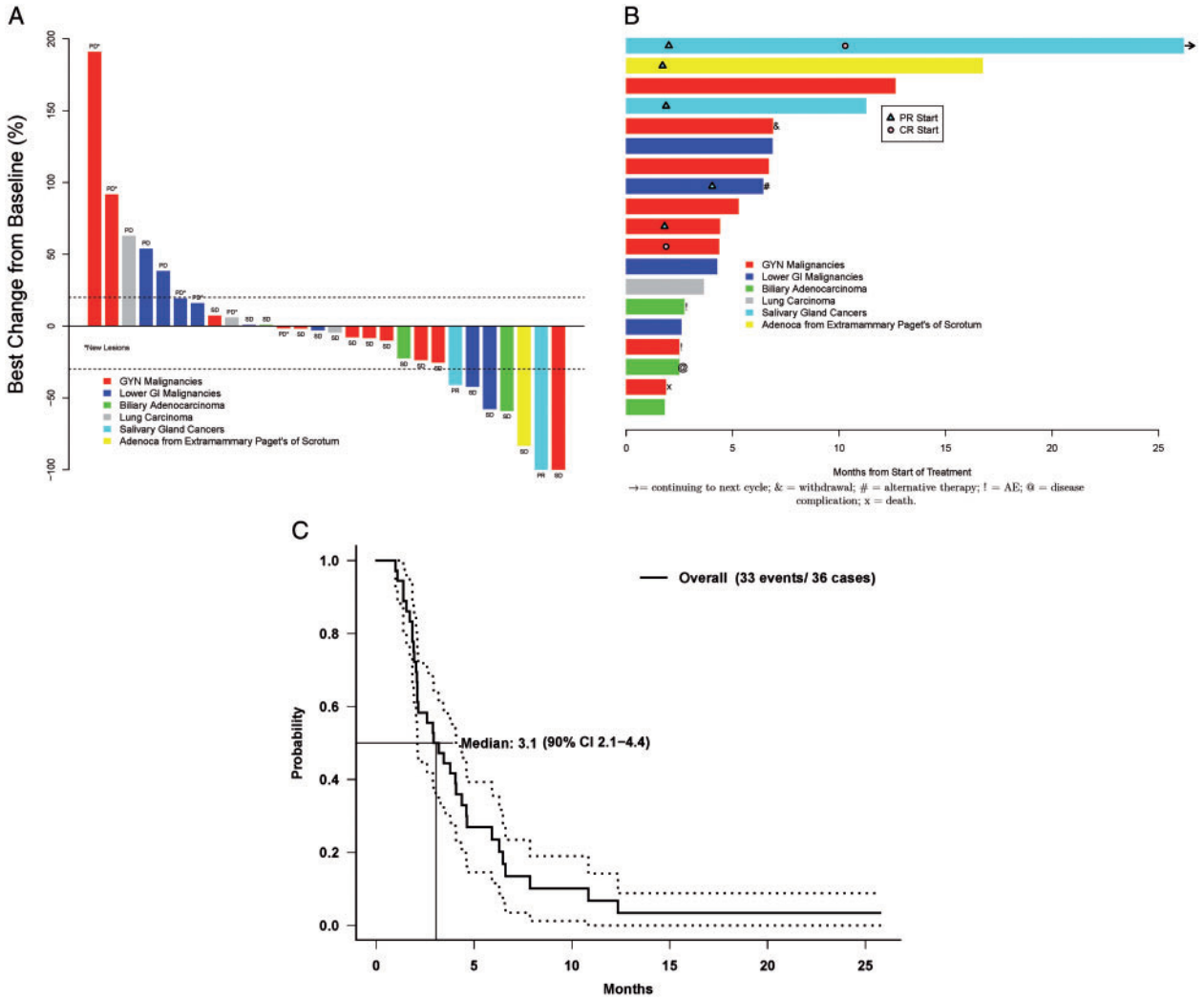

Figure 3. (A) Efficacy: Best% change from baseline ($n = 28$). Four patients had PD based on new lesions but data for the target lesions are not available so not included in this plot. (B) Treatment Duration in patients who achieved SD or PR. (C) Kaplan–Meier curve for progression-free survival. GYN, gynecological; CI, confidence interval; GI, gastrointestinal.

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Table 2. Treatment-related toxicities in patients who started treatment (n = 38)

Adverse event	Grade 1, 2 (%)	Grade 3 (%)	Grade 4 (%)
Anemia	7 (18)	3 (8)	
Chills	2 (5)		
Fatigue	13 (34)	2 (5)	
Fever	3 (8)	1 (3)	
Gait disturbance	2 (5)		
Limb edema	3 (8)		
Acneiform rash	3 (8)		
Maculo-papular rash	2 (5)		
Constipation	2 (5)		
Dry mouth	2 (5)		
Oral mucositis	2 (5)		
Nausea	8 (21)	1 (3)	
Vomiting	8 (21)		
Ileal obstruction		1 (3)	
Allergic reaction	2 (5)		
Alanine aminotransferase Increase	4 (11)		
Alkaline phosphatase Increase	5 (13)	1 (3)	
Aspartate aminotransferase Increase	10 (26)	1 (3)	
Bilirubin increase	3 (8)		
Creatinine increase	3 (8)		
Lymphocyte count decrease	2 (5)	1 (3)	
Neutrophil count decrease	3 (8)	1 (3)	
Platelet count decrease	10 (26)	2 (5)	
Weight loss	3 (8)		
White blood cell decrease	4 (11)		
Anorexia	5 (13)	1 (3)	
Myalgia	2 (5)		
Generalized muscle weakness	3 (8)		
Headache	4 (11)		
Peripheral motor neuropathy	2 (5)		
Peripheral sensory neuropathy	4 (11)		
Cough	3 (8)		
Epistaxis	3 (8)	1 (3)	
Hypoxia		1 (3)	
Muscle weakness lower limb		1 (3)	
Dehydration		2 (3)	
Investigations: Other, specify	1 (3)	1 (3)	
Urinary tract infection		1 (3)	
Upper respiratory infection		1 (3)	
Diarrhea		1 (3)	
Blurred vision		1 (3)	
Total worst degree	20 (58)	11 (16)	0 (0)

Includes specific adverse events with at least two grade 1 or 2 events or 1 grade 3 event. Total worst degree includes all treatment-related adverse events.

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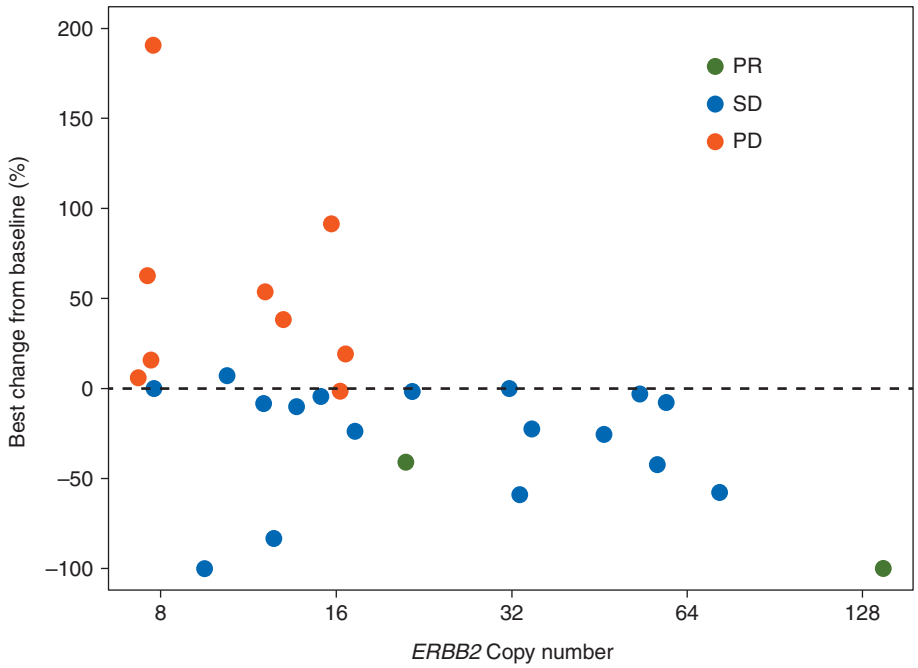

Figure 4. Correlation of best % change by *ERBB2* copy number gain ($n = 28$). PR, partial response; SD, stable disease; PD, progressive disease.

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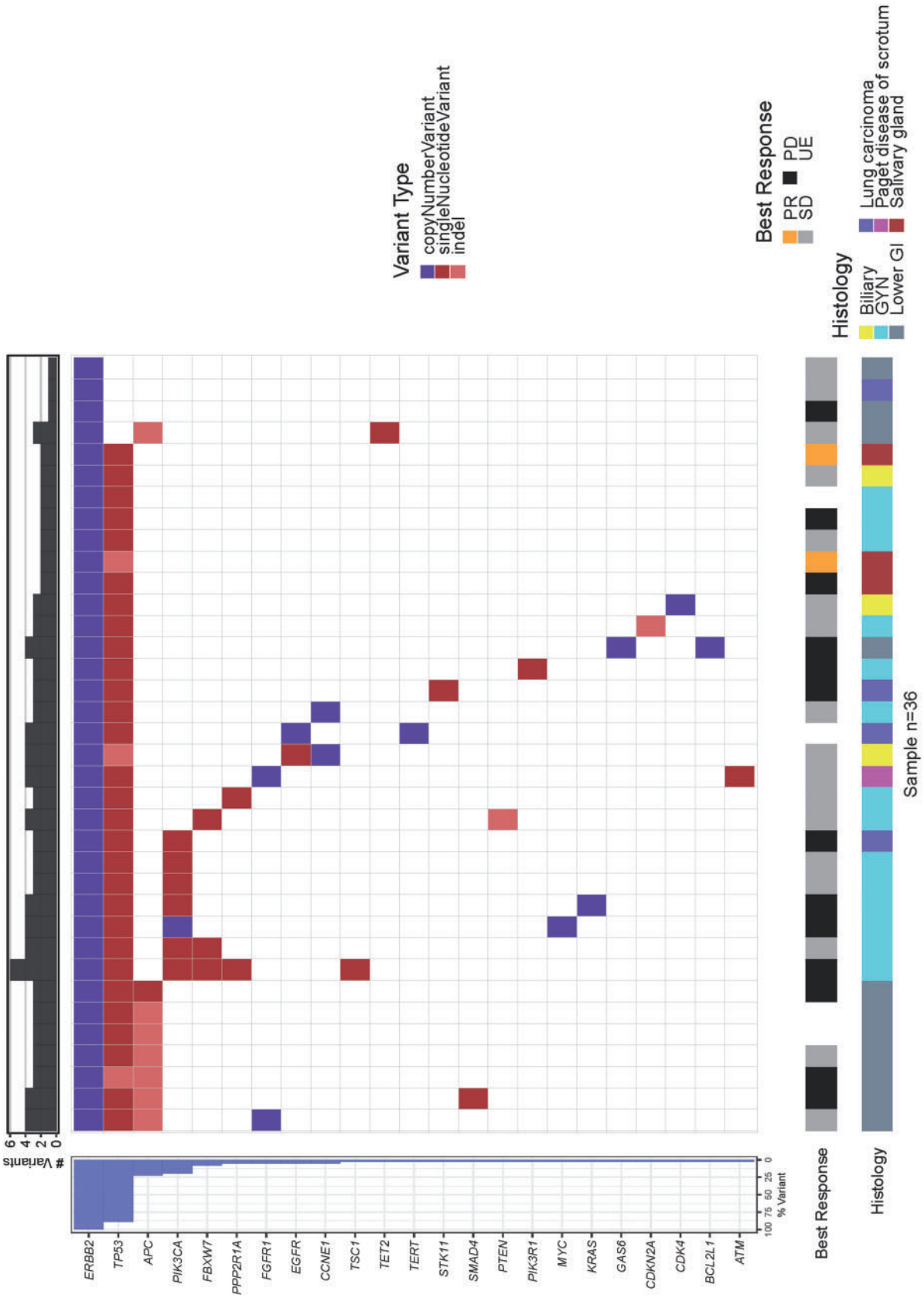

Figure 5. Co-occurring genomic alterations with *HER2* amplification using the NCI-MATCH assay color coded by variant type (left): copy number variant (purple), single nucleotide variant (red), indel (pink) and number of variants (top) for each eligible patient along with information regarding histology and best response on treatment (bottom): partial response (PR), stable disease (SD), progression of disease (PD), unevaluable (UE).

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