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Targeting Chromosomal Instability and Tumour Heterogeneity in HER2-Positive Breast Cancer

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ABSTRACT

Chromosomal instability (CIN) is a common cause of tumour heterogeneity and poor prognosis in solid tumours and describes cell–cell variation in chromosome structure or number across a tumour population. In this article we consider evidence suggesting that CIN may be targeted and may influence response to distinct chemotherapy regimens, using HER2-positive breast cancer as an example. Pre-clinical models have indicated a role for HER2 signalling in initiating CIN and defective cell-cycle control, and evidence suggests that HER2-targeting may attenuate this process. Anthracyclines and platinum agents may target tumours with distinct patterns of karyotypic complexity, whereas taxanes may have preferential activity in tumours with relative chromosomal stability. A greater understanding of karyotypic complexity and identification of methods to directly examine and target CIN may support novel strategies to improve outcome in cancer. J. Cell. Biochem. 111: 782–790, 2010.

KEY WORDS: CHROMOSOMAL INSTABILITY; HER2; BREAST CANCER; DRUG RESISTANCE; TUMOUR HETEROGENEITY; MICROTUBULE; CEP17

Most cancers are of monoclonal origin, but growth of cellular sub-populations occurs during progression and leads to intra-tumour genetic heterogeneity [Shah et al., 2009; Ding et al., 2010]. Genomic instability promotes cell-to-cell variability in the genomic content of cells across a tumour population. From a Darwinian standpoint, this may enhance the propensity for tumours to 'adapt' to environmental stress through selection [Cahill et al., 1999]. Genomic instability can occur at a DNA sequence level (e.g. microsatellite instability, commonly seen in colorectal cancers) or may be evident at the gross karyotypic level (aneuploidy). Furthermore, new patterns of genetic instability are emerging from massively parallel sequencing studies [Stephens et al., 2009]. Aneuploidy refers to the state of abnormal chromosome number or structure, and can be either stable or unstable. Unstable aneuploidy (or chromosomal instability, CIN), leads to karyotypic heterogeneity between tumour cells [Geigl et al., 2008].

CIN is associated with poor prognosis in solid tumours, including breast cancer [Carter et al., 2006; Walther et al., 2008], which may be explained by the consequences of CIN on tumour adaptation and evolution, and an accelerated capacity to acquire multi-drug resistance and adaptation to environmental stress compared to chromosomally stable, diploid cells [Cahill et al., 1999; Duesberg et al., 2000; McClelland et al., 2009]. Consistent with this hypothesis, mouse lung tumours driven by KRAS have a higher tumour relapse rate following oncogene withdrawal in the presence of MAD2-driven CIN, than tumours with KRAS expression alone [Sotillo et al., 2010].

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Evidence accumulated over the last decade suggests that tumour CIN may be an attractive and targetable phenotype due to its almost exclusive predominance in neoplastic compared to normal tissue. Roschke and colleagues were amongst the first to suggest that tumour karyotypic complexity may be an exploitable phenotype and defined several anticancer agents with preferential activity in chromosomally unstable cell lines [Roschke et al., 2003, 2005; Roschke and Kirsch, 2005]. However, one of the remaining challenges in the targeting of this pattern of genome instability is the ability to determine CIN status in tumour tissue. The best current measures of CIN quantify inter-cellular heterogeneity [Geigl et al., 2008]; fluorescence in situ hybridisation (FISH) allows assessment of intercellular variation in chromosome number [Lingle et al., 2002] and DNA image cytometry measures heterogeneity in nuclear DNA content and can serve as a direct measure of aneuploidy and CIN [Kronenwett et al., 2004]. Next generation sequencing technologies have the capacity to address the diversity of genomic aberrations and define tumour cell heterogeneity. In addition, coordinated aberrations in gene expression can be used to indicate chromosomal imbalances, a measure termed 'total functional aneuploidy' (tFA) [Carter et al., 2006]. The CIN70 gene expression signature, expression of which is highly correlated with tFA, is predictive of poor outcome in six different cancer types including breast cancer [Carter et al., 2006]. Importantly, CIN70 expression correlates closely with CIN quantified by DNA image cytometry in breast cancer, confirming CIN70 expression as a robust surrogate marker of CIN in vivo [Swanton et al., 2009].

In this article we review evidence for the occurrence of CIN in HER2+ breast cancer and consider the hypothesis that tumour heterogeneity and CIN may influence response profiles observed in clinical trials in this disease subtype. We examine a model supported by recent clinical and molecular evidence that response to combination chemotherapy given with HER2-targeted therapies may reflect the differential targeting of karyotypically distinct tumour populations by these agents.

IMPACT OF HER2 TARGETING ON CELL-CYCLE CONTROL AND CHROMOSOMAL INSTABILITY

HER2, human epidermal growth factor receptor 2, is amplified and overexpressed (Fig. 1) in up to 15% of breast cancers, and is associated with poor prognosis [Slamon et al., 1987]. The anti-HER2 monoclonal antibody, trastuzumab, improves outcome in HER2-positive (HER2+) primary and metastatic breast cancer [Slamon et al., 2001; Mariani et al., 2009]. Trastuzumab has multiple potential mechanisms of action, including immune activation and inhibition of signalling pathways that may be essential for maintaining cancer cell viability and CIN [Hudis, 2007; Valabrega et al., 2007].

HER2+ breast cancers show significantly increased allelic imbalance and CIN relative to HER2-negative tumours [Ellsworth et al., 2008]. HER2 overexpression in tumours is also associated with centrosome abnormalities [Montagna et al., 2002; Schneeweiss et al., 2003], which may promote CIN through chromosome missegregation during mitosis [Ganem et al., 2009]. In addition,

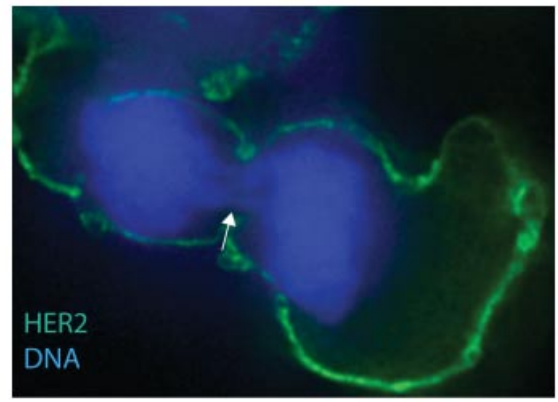

Fig. 1. CIN breast cancer cells missegregate chromosomes at mitosis: HER2-positive BT474 breast cancer cell completing mitosis with a chromosome segregation error (DNA in the cytokinesis furrow is indicated by arrow heads). HER2 is stained with an anti-HER2 antibody and is shown in green. DNA is stained with DAPI and is shown in blue. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

there are several lines of pre-clinical and clinical evidence to support a functional role of HER2 signalling in mediating and sustaining CIN. HER2 protein overexpression constitutively activates the PI3K/AKT/mTOR pathway, and activity of this signalling axis promotes chromosome segregation errors in mouse tumours [Aoki et al., 2003] and the survival and proliferation of aneuploid cells in culture [Jin and Woodgett, 2005; Wang et al., 2006]. The tumour suppressor PTEN antagonises PI3K activity, and like HER2 overexpression, loss of PTEN results in constitutive AKT activation. PTEN loss is common both in mouse tumour models of CIN [Maser et al., 2007], and in aneuploid human tumours [Ehlers et al., 2008; Li et al., 2009]. Recently it has been established that PTEN loss results in a substantial reduction in the ability of cells to elicit RAD51 foci formation and homologous recombination DNA repair further contributing to CIN [Mendes-Pereira et al., 2009].

Consistent with a role for HER2 signalling in driving CIN, silencing HER2 expression results in the selective apoptosis of cancer cells with an abnormal karyotype and the specific regression of the aneuploid fraction [Pack et al., 2004]. Furthermore, inhibition of either PI3K or mTOR reduces the frequency of chromosome segregation errors [Aoki et al., 2003].

The impact of trastuzumab exposure on cell-cycle progression may help to explain the restoration of chromosomal stability mediated by HER2 targeting in vitro (Fig. 2) [Pack et al., 2004]. Trastuzumab exposure results in the repression of genes encoding components of the mitotic apparatus, including genes with direct roles in centrosome integrity, spindle and kinetochore attachments and spindle assembly checkpoint function [Le et al., 2006]. Several of these genes, for example, Aurora kinase A [Anand et al., 2003], HEC1 [Diaz-Rodriguez et al., 2008] and UBE2C [Reddy et al., 2007], are implicated in CIN directly and half of the genes consistently repressed by trastuzumab are overexpressed in the CIN70 signature ($P = 2.3 \times 10^{-16}$) [Carter et al., 2006; Le et al., 2006; Swanton et al., 2007]. Trastuzumab may also restore chromosomal stability by

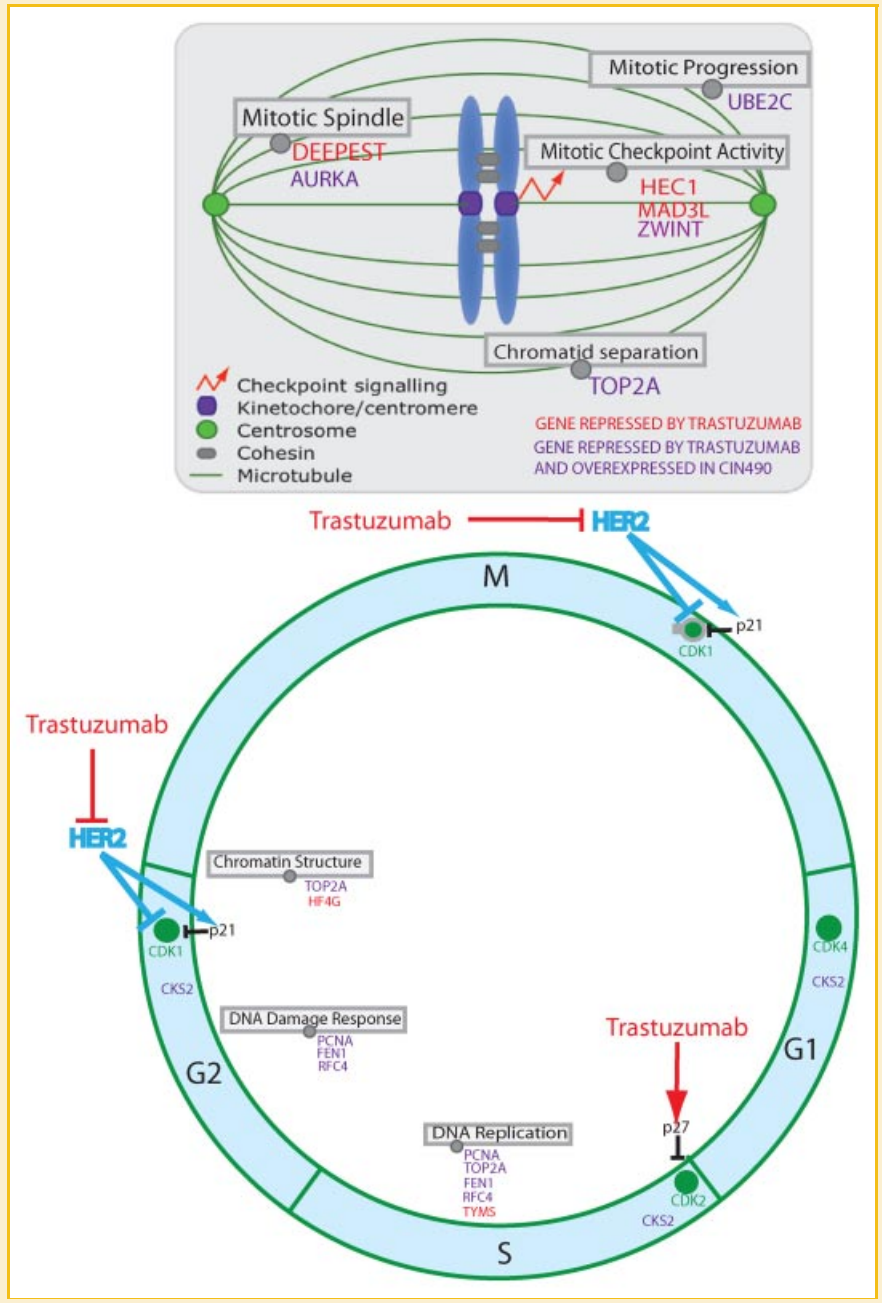

Fig. 2. HER2 targeting by trastuzumab regulates genes involved in cell-cycle progression and mitosis. HER2 signalling leads to inhibitory phosphorylation of CDK1, and upregulation of $p21^{cip1}$. Trastuzumab treatment reverses this effect and increases the pool of $p27^{kip1}$-bound CDK2, which controls entry into S-phase. Trastuzumab treatment also regulates cell-cycle genes through transcriptional repression. Genes repressed by trastuzumab are shown in red. Eight out of 16 of these genes are overexpressed in CIN tumours (shown in purple). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

altering cyclin-dependent kinase (CDK) activity. In mitosis, the spindle assembly checkpoint sustains activity of the master mitotic kinase CDC2 (CDK1), and maintains sister chromatid cohesion in order to delay progression through mitosis until all chromosomes are correctly aligned and attached to the mitotic spindle at metaphase. HER2 overexpression inhibits CDC2 activity through upregulation of the cyclin/CDK inhibitor p21Cip1 [Yu et al., 1998], and through inhibitory phosphorylation of CDC2 [Tan et al., 2002].

Trastuzumab's impact on cell-cycle checkpoint control mechanisms may directly affect chemotherapy responsiveness, since decreased CDC2 activity is associated with resistance to paclitaxel-induced apoptosis in vitro [Yu et al., 1998; Tan et al., 2002]. Treatment of HER2+ breast cancer cell lines with trastuzumab leads to repression of p21cip1 expression and reduction in the inhibitory phosphorylation state of CDC2, thereby augmenting CDC2 activity and paclitaxel-induced cell death [Lee et al., 2002]. Inhibition of

CDC2 by increased HER2 signalling may bypass spindle assembly checkpoint activation, which normally acts to maintain CDC2 activity, potentially facilitating chromosome missegregation events during mitosis. Therefore, trastuzumab treatment reactivates CDC2 at mitosis, potentiating checkpoint signalling that may result in both enhanced chromosomal stability and taxane responsiveness [Yu et al., 1998; Tan et al., 2002; Le et al., 2006].

TUMOUR HETEROGENEITY IN HER2-POSITIVE BREAST CANCER

HER2 expression is heterogeneous in HER2+ breast cancer; variation in HER2 amplification and expression exists both in HER2+ cell lines and within individual tumours [Szollosi et al., 1995; Lewis et al., 2005; Shin et al., 2006; Moeder et al., 2007; Brunelli et al., 2009]. For a tumour to be classified as HER2+, 30% of tumour cells must show strong membrane staining by immunohistochemistry (IHC 3+) implying that intra-tumour heterogeneity in HER2 expression occurs in HER2+ disease. Intra-tumour heterogeneity of HER2 amplification has been documented in HER2 IHC 3+ cancers: 30% of tumours with high-grade gene amplification (HER2 copy number ratio $\geq$4.0) demonstrated areas with low-grade amplification (HER2 copy number ratio $>$2.2 to $<$4), and 40% of those with low-grade amplification demonstrated areas of no amplification [Brunelli et al., 2009]. Intra-tumour heterogeneity of both HER2 amplification and expression has also been observed in IHC 2+ breast cancers [Lewis et al., 2005]. In agreement with a role for HER2 signalling in the development of CIN and consistent with the heterogeneity of CIN in individual tumours, flow cytometry experiments have indicated that the expression of HER2 is higher in aneuploid compared to diploid components of HER2+ tumours. Furthermore, the acquisition of HER2 overexpression may precede the initiation of aneuploidy [Smith et al., 2000]. Intra-tumour heterogeneity in HER2 status may therefore initiate heterogeneity of CIN, with higher HER2 expression/amplification in aneuploid/CIN cells.

The presence of CIN is not a universal karyotypic feature across all breast cancers. Direct measurements of CIN quantified by DNA image cytometry, or surrogate quantification of CIN70 signature expression, have determined that breast cancers expressing HER2 do not all have evidence of CIN. In a small cohort of primary breast cancers with paired gene expression and DNA image cytometry data approximately one-third of HER2-positive tumours displayed evidence of chromosomal stability (as defined by DNA image cytometry and CIN70 signature expression) [Habermann et al., 2009; Swanton et al., 2009]. We have validated these findings in a meta-analysis of 1,850 breast cancer patients, and found that 32–41% of HER2+ tumours display low expression of the CIN70 signature (Table II) indicative of relative chromosomal stability.

In summary, HER2+ breast cancer is a heterogeneous disease subtype, and the presence of CIN may be heterogeneous in HER2+ disease both within and between tumours that may have therapeutic implications. We now consider the evidence that chemotherapy agents used in HER2-positive breast cancer may target karyotypically distinct tumour subpopulations within this heterogeneous disease type.

ANTHRACYCLINES AND PLATINUM AGENTS

Clinical trials have demonstrated an increased frequency of pathological complete response in HER2-positive primary breast cancer, and improved tumour response rates in metastatic disease, when trastuzumab is combined with anthracycline-containing regimens [Slamon et al., 2001; Pritchard et al., 2008].

Recently, much attention has focused on HER2, TOP2A and structural instability of the peri-centromeric region of chromosome 17 as potential predictive markers of benefit from anthracyclines, which inhibit the activity of topoisomerase II [Tubbs et al., 2009; Gennari et al., 2008; Pritchard et al., 2006]. TOP2A, which encodes topoisomerase II, and HER2 are encoded on neighbouring sites near the peri-centromeric region of the long arm of chromosome 17. Increasing evidence suggests that TOP2A amplification may be associated with response or preferential benefit from anthracyclines [Isola et al., 2000; Knoop et al., 2005; Slamon, 2006; O'Malley et al., 2009]. Recently, structural CIN around the centromeric region of chromosome 17 has been associated with anthracycline benefit. Using CGH (comparative genomic hybridisation) or MLPA (multiplex ligation-dependent probe amplification) approaches, three groups have shown increased Centromeric Enumeration Probe 17 (CEP17) copy number reflects complex structural instability of chromosome 17 and amplification of the centromeric region of chromosome 17, rather than gain of the whole chromosome [Marchio et al., 2009; Moelans et al., 2009; Yeh et al., 2009]. Tumours with CEP17 duplication may be associated with preferential sensitivity to anthracycline-based regimens. Four studies, including three adjuvant clinical trials (NEAT, BR9601 and Canadian MA.5), have documented improved disease free survival in patients with primary breast tumours with CEP17 duplication, following anthracycline combination chemotherapy [Bartlett et al., 2009, 2010]. Analysis of one of these studies, the MA.5 trial, indicated that benefit from anthracyclines was also associated with HER2 and TOP2A amplification [O'Malley et al., 2009]. Conceivably underlying structural CIN of chromosome 17 may be a surrogate for preferential benefit from anthracycline therapy due to structural instability of the TOP2A locus adjacent to the CEP17 centromeric region.

Preliminary evidence also suggests that platinum chemotherapy agents may be preferentially active in tumours with structural CIN. Genome-wide measures of structural CIN classified by DNA copy number analysis (fractional allelic loss FAL) or by the CIN70 gene expression signature correlating with tFA or by the total number of DNA breakpoints, have determined that high tFA, FAL or total DNA breakpoint number, may be associated with preferential sensitivity to platinum-based therapies in gastric, ovarian and breast cancer [Ott et al., 2003; Juul et al., 2009; Swanton et al., 2009]. Our data indicate that in contrast to ER-/PR-/HER2-negative breast cancer where the majority of tumours display high tFA/CIN70 expression, the distribution of high and low tFA in HER2-positive disease appears bimodal with approximately 32–41% of HER2-positive breast cancers demonstrating low tFA/CIN70 expression (Table II) that might be predicted to be less sensitive to platinum therapy.

It should also be considered that the underlying genomic aberrations responsible for the CIN state may directly influence

chemotherapy response and thus CIN and treatment response may only be associated via an underlying tumour somatic event that results in both instability and enhanced chemosensitivity. For example, PTEN loss that occurs in a subset of HER2-positive breast cancers, may promote chromosome segregation errors through activation of PI3K-AKT pathway signalling and defective homologous recombination resulting in enhanced platinum sensitivity [Aoki et al., 2003; Mendes-Pereira et al., 2009].

In summary, evidence suggests that both anthracyclines and platinum agents have preferential activity in tumours with defined patterns of karyotypic complexity. If this is the case, one might predict that platinum agents may be able to substitute for anthracyclines in HER2-positive breast cancer, thereby limiting the cardiotoxicity profile of anthracycline-based therapy. Consistent with this hypothesis, the BCIRG006 study has documented equivalent disease free survival in an adjuvant HER2-positive breast cancer clinical trial with a platinum-containing regimen (docetaxel/carboplatin/trastuzumab) compared to an anthracycline-based regimen (adriamycin/cyclophosphamide/docetaxel/trastuzumab) [Slamon, 2006].

TAXANES

The ability to survive an aneuploid state correlates with resistance to microtubule inhibitor drugs, leading to suggestions that the development of polyploidy in response to paclitaxel may be a useful indicator of drug resistance [Roberts et al., 1990; Bouchet et al., 2007]. This has led to the proposition that tumour CIN status may influence sensitivity to microtubule stabilising agents in vivo [Weaver and Cleveland, 2005]. Evidence substantiating this association derives from studies demonstrating that many genes implicated in resistance to microtubule inhibitors have roles in the maintenance of chromosomal stability [Cahill et al., 1998; Michel et al., 2001; Anand et al., 2003; Hauf et al., 2003; Sudo et al., 2004; Wang et al., 2004; Schmidt et al., 2005; Chabalier et al., 2006; Andre et al., 2007; Pusztai, 2007] and an efficient mitotic arrest orchestrated by the spindle assembly checkpoint, which monitors chromosome attachment to the mitotic spindle and sustains faithful chromosome segregation and chromosomal stability, is required for paclitaxel-induced cell death [Sudo et al., 2004; Juul et al., 2010].

Consistent with a role for an aneuploid/CIN survival state in taxane resistance, CIN is associated with taxane resistance in vitro [Bouchet et al., 2007; Swanton et al., 2007] and predicts intrinsic taxane resistance in patients in vivo [Swanton et al., 2009]. Clinical trials in advanced breast cancer have consistently shown that trastuzumab or lapatinib therapy in combination with taxanes are associated with additive clinical benefit in patients with HER2+ disease when compared to the expected monotherapy response rates of either trastuzumab or lapatinib [Vogel et al., 2002; Baselga et al., 2005] or taxane alone (Table I) [Slamon et al., 2001; Marty et al., 2005; Romond et al., 2005; Gasparini et al., 2007; Di Leo et al., 2008; Gomez et al., 2008]. Additive response rates witnessed in these studies raise the possibility that taxanes and HER2 directed therapies target distinct tumour populations that can be explained by inter- or intra-tumour variation in CIN. Not all HER2+ breast tumours appear

TABLE I. Trastuzumab/Lapatinib Monotherapy Clinical Trials and Combination Therapy Trial Data With Taxanes and/or Anthracyclines

Study	Patients	Drug regimen	Previous Chemotherapy	Response Rate			
				Trastuzumab/ lapatinib monotherapy	Chemotherapy alone	Combination therapy prediction = (ORR (trastuzumab/lapatinib monotherapy) + ORR (chemotherapy alone))	Combination therapy
Baselga et al. [2005]	n = 105	Trastuzumab	72% neo/adjuvant (49% anthracyclines)	ORR 23%			
Vogel et al. [2002]	n = 114	Trastuzumab	68% adjuvant (50% anthracyclines)	ORR 26%			
Marty et al. [2005]	n = 186	Docetaxel or docetaxel + trastuzumab	69·5% adjuvant (59·5% anthracyclines)		ORR 34%	58·5%	ORR 61%
Gasparini et al. [2007]	n = 118	Paclitaxel or paclitaxel + trastuzumab	63% adjuvant (56·9% anthracyclines)		ORR 56·9%	81·4%	ORR 75%
Slamon et al. [2001]	n = 188	Paclitaxel (n = 93) or paclitaxel + trastuzumab (n = 92)	98·5% adjuvant anthracyclines		ORR 17%	41·5%	ORR 41%
	n = 281,	Anthracyline (doxorubicin or epirubicin) cyclophosphamide ± trastuzumab	46% adjuvant (non-anthracycline)		ORR 42%	66·5%	ORR 56%
Gomez et al. [2008]	n = 138	Lapatinib	Neoadjuvant (10·5%), adjuvant (38%)	ORR 24%			
Di Leo et al. [2008]	n = 86	Paclitaxel (n = 288) or paclitaxel + lapatinib (n = 291)	Adjuvant taxanes (7%), adjuvant anthracyclines (44%)		ORR 37·8%	61·8%	ORR 63·3%

ORR, objective response rate according to RECIST criteria (the percentage of patients showing either tumour shrinkage (at least 30%) or complete disappearance of all target tumour lesions).
All studies include patients who have not previously received treatment for metastatic breast cancer.

TABLE II. Distribution of High and Low CIN Tumours in HER2-Positive Disease

	IHC/FISH			Expression		
	HER2 positive	HER2 negative	Total with IHC/FISH	HER2 positive	HER2 negative	All samples
CIN high	79 (59%)	223 (48%)	302 (51%)	210 (68%)	687 (45%)	897 (48%)
CIN low	54 (41%)	240 (52%)	294 (49%)	101 (32%)	852 (55%)	953 (52%)
Total	133 (100%)	463 (100%)	596 (100%)	311 (100%)	1,539 (100%)	1,850 (100%)

CIN high and CIN low status is determined for each of the 1,850 samples, derived from published gene expression datasets from 10 breast cancer cohorts (mixed histopathological subtypes), as the relative expression of the CIN70 signature to the mean CIN70 expression across the whole cohort. HER2 status is determined by both IHC/FISH (available for 596 samples) and by expression of the HER2 gene (inferred on all 1,850 samples). HER2 status by expression was inferred by observing the cumulative density function of HER2 expression in a mixed cohort. HER2-positive tumours were identified by a discontinuous point separating high HER2 expressing tumours from low. Numbers represent individual patients. The distribution of CIN high versus CIN low for each column is given in percent in parenthesis.

to be highly chromosomally unstable as defined by the CIN70 expression signature (Table II) and heterogeneous HER2 expression is associated with differential karyotypic instability within the tumour population indicative of inter and intra-tumoural CIN heterogeneity, respectively [Smith et al., 2000].

Evidence reviewed in this article indicate that taxanes may provide greater benefit in patients with chromosomally stable tumours while HER2 targeting limits the development of aneuploid cells [Pack et al., 2004]. Hence trastuzumab and taxanes may target karyotypically distinct subpopulations within the same tumour [Pack et al., 2004; Le et al., 2005]. Trastuzumab combination treatment (which decreases inhibitory CDC2 phosphorylation) may also enhance paclitaxel-induced cell death through enhanced CDC2 activity following trastuzumab exposure [Lee et al., 2002]. Trastuzumab may therefore potentiate paclitaxel cytotoxicity by strengthening spindle assembly checkpoint signalling in CIN cells, further enhancing the combined efficacy of these two treatment modalities.

In the absence of knowledge of tumour CIN status before treatment and taking into account evidence that some HER2-positive breast cancers may display relative chromosomal stability, cytotoxic agents targeting CIN and non-CIN tumours would be required to achieve maximum benefit across all patients with HER2-positive breast cancer. Taxane combination regimens with platinum or anthracycline would be predicted to be optimal in order to target both karyotypically stable or unstable tumours. Consistent with this concept, the CALGB-9344 clinical trial demonstrated that patients with surgically resected HER2-positive breast cancer derived preferential benefit from the combination of anthracycline with paclitaxel chemotherapy [Hayes et al., 2007] and a recent pre-operative chemotherapy trial demonstrated high pathological complete response rates (76%) in HER2-positive breast cancer treated with paclitaxel/carboplatin and trastuzumab [Sikov et al., 2009].

CONCLUDING REMARKS

Evidence is emerging that activity of individual cytotoxic agents may be influenced by the underlying tumour karyotypic complexity and patterns of genetic instability. Such observations may be correlative and attributable to the underlying DNA repair or chromosome segregation defects within the tumour. The distribution of CIN in HER2-positive breast cancer may provide a rational basis to understand the role of individual cytotoxic therapies in this disease. It is possible that anthracyclines and platinum agents may target more karyotypically complex tumours, in contrast to taxanes where pre-clinical and clinical data suggest increased activity in tumours with chromosomal stability. Based on these data, novel combinatorial strategies targeting tumour karyotype and CIN might be considered to target this high-risk cancer phenotype.

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