

**UNIVERSIDAD PEDAGÓGICA Y TECNOLÓGICA DE COLOMBIA
FACULTAD DE CIENCIAS
ESCUELA DE CIENCIAS BIOLÓGICAS-POSGRADO
MAESTRÍA EN CIENCIAS BIOLÓGICAS**

**IMPLICACIONES DE LA INESTABILIDAD CROMOSÓMICA Y HETEROGENEIDAD
CLONAL EN LA RESPUESTA A LA TERAPIA EN CÁNCER DE SENO**

Requisito para optar el título de Magister en Ciencias Biológicas

INGRID NATALIA VARGAS RONDÓN

Tunja
Octubre, 2019

**UNIVERSIDAD PEDAGÓGICA Y TECNOLÓGICA DE COLOMBIA
FACULTAD DE CIENCIAS
ESCUELA DE CIENCIAS BIOLÓGICAS-POSGRADO
MAESTRÍA EN CIENCIAS BIOLÓGICAS**

IMPLICACIONES DE LA INESTABILIDAD CROMOSÓMICA Y HETEROGENEIDAD CLONAL EN LA RESPUESTA A LA TERAPIA EN CÁNCER DE SENO

Requisito para optar el título de Magister en Ciencias Biológicas

INGRID NATALIA VARGAS RONDÓN

DIRECTORA
SANDRA MILENA RONDÓN LAGOS MSc, Ph.D
Universidad Pedagógica y Tecnológica de Colombia
Grupo de investigación en Ciencias Biomédicas (GICBUPTC)

CODIRECTORA
VICTORIA EUGENIA VILLEGRAS GÁLVEZ MSc, Ph.D
Universidad del Rosario
Grupo de investigación en Moléculas Biológicas y Actividad Celular

Tunja
Octubre, 2019

CERTIFICADO DE ORIGINALIDAD

Sandra Milena Rondón Lagos, **Doctora en Ciencias Biomédicas, docente de la Universidad Pedagógica y Tecnológica de Colombia** y Victoria Eugenia Villegas Gálvez, **Doctora en Ciencias Biomédicas, docente de la Universidad del Rosario.**

CERTIFICAMOS:

Que el trabajo de grado realizado bajo nuestra dirección por **Ingrid Natalia Vargas Rondón** titulado “**IMPLICACIONES DE LA INESTABILIDAD CROMOSÓMICA Y HETEROGENEIDAD CLONAL EN LA RESPUESTA A LA TERAPIA EN CÁNCER DE SENO**”, reúne las condiciones de originalidad requeridas para optar al título de **Magister en Ciencias Biológicas** otorgado por la **Universidad Pedagógica y Tecnológica de Colombia**.

Y para que así conste, firmamos la siguiente certificación en Tunja, octubre de 2019.

Sandra Milena Rondón Lagos. MSc, Ph.D

Director

Universidad Pedagógica y Tecnológica de Colombia

Grupo de investigación en Ciencias Biomédicas (GICBUPTC)

Victoria Eugenia Villegas Gálvez. MSc, Ph.D

Codirector

Universidad del Rosario

Grupo de investigación en Moléculas Biológicas y Actividad Celular.

A Dios por permitirme llegar hasta acá.

A mis papás. No tengo como agradecerles todo lo que hacen por mí, no tengo palabras suficientes para decirles que son mi razón de vivir. A ellos, por ser mi refugio seguro, por ser los seres más incondicionales y fieles, por entregar toda su vida a mí, por dejar de cumplir sus sueños y ayudarme a cumplir los míos, por ser mi ejemplo de fortaleza y valentía. Por ser mi soporte y más grande motivación.

A mi hermana. Por ser mi ejemplo de fortaleza y perseverancia. Porque no importa si las circunstancias no juegan a nuestro favor, si hay esfuerzos en vano o angustias que parezcan asfixiarnos. Si seguimos soñando, esperando y sonriendo juntas, siempre llegaremos lejos.

A mi familia, por ser mis ángeles acá en la tierra. Por ser la muestra de amor e incondicionalidad más grande de la vida.

A mi novio, por su apoyo y fortaleza, por creer siempre en mí. Por estar conmigo en los momentos buenos que nos llenaron de esperanzas y por todos los malos que nos hicieron fuertes. Por todas las circunstancias que nos hicieron el camino más difícil, y por otras que como hoy nos demuestras que todo, absolutamente todo, vale la pena.

Agradecimientos

A la Universidad Pedagógica y Tecnológica de Colombia y al grupo de investigación en Ciencias Biomédicas (GICBUPTC), por abrirme las puertas y brindarme soporte profesional y logístico para alcanzar los objetivos perseguidos. Muchas gracias por permitirme vivir una experiencia tan importante para mi formación como investigador.

A la Universidad del Rosario y al grupo de investigación Moléculas Biológicas y Actividad Celular, por todas las facilidades otorgadas, por la ayuda incondicional en todo lo relacionado con la ejecución de la investigación y por acogerme como un miembro más de su institución. Su colaboración fue de gran ayuda durante mi estancia en su laboratorio.

A la convocatoria DIN14 Cooperación Institucional UPTC 12-07-2017 por su apoyo económico para realizar parte de esta investigación.

A los estudiantes del Semillero de Investigación en Ciencias Básicas y Aplicadas, por su disposición y compromiso con el aprendizaje.

A la Dra. Ruth Maribel Forero, por abrirme las puertas en su grupo de investigación y por su disposición para colaborar siempre.

Al Dr. Nelson Rangel, por su paciencia, disponibilidad y generosidad para compartir su experiencia y amplio conocimiento, también por sus siempre atentas y rápidas respuestas a las diferentes inquietudes que surgieron durante el desarrollo de este trabajo.

A la Dra. Victoria Villegas, por su apoyo y motivación para culminar mis estudios de maestría. Por su disposición y compromiso a formarme no solo como profesional, sino también como persona. Porque nunca escatimó esfuerzo y tiempo para corregir y mejorar este trabajo, por sus valiosos conocimientos, y su correcta orientación, por ser firme en los momentos que se requería; pero sobre todo, porque durante todo el proceso me demostró que aparte de ser una gran profesora, es excelente ser humano.

Un trabajo de investigación es siempre fruto de ideas, proyectos y esfuerzos previos que corresponden a otras personas. En este caso mi más sincero agradecimiento a la Dra. Milena Rondón, con quien estaré siempre en deuda. Gracias por su amabilidad, su tiempo, su gran conocimiento y apoyo incondicional, por su constante interés en infundirme la investigación como parte esencial del aprendizaje. Gracias por creer en mí y convertirse en mi ejemplo a seguir. A lo largo de mi carrera, me he encontrado con profesores que me han transmitido conocimientos importantes, los cuales me han ayudado a llegar hasta acá; sin embargo, de ninguno aprendí el amor y la pasión para realizar algo, como lo aprendí de ella, y sin pensarlo me enseñó que más que conocimiento, hay que trasmisitir sentimiento, aprender a amar y enamorarse de lo que uno hace, apasionarse por el proceso y jamás rendirse y eso, junto con la dedicación y la constancia es el resultado del éxito. Gracias infinitas porque gracias a usted hoy puedo decir que soy mejor profesional y mejor persona.

TABLA DE CONTENIDO

	Pág
RESÚMEN	1
CAPITULO I. GENERALIDADES	2
1. INTRODUCCIÓN	3
2. MARCO CONCEPTUAL	4
2.1 Generalidades del Cáncer	4
2.2 Cáncer de Seno	4
2.2.1 Epidemiología	5
2.2.2 Clasificación del Cáncer de Seno	5
2.2.3 Tratamientos contra el Cáncer de Seno	6
2.2.3.1 Tratamiento Hormonal	6
2.2.3.2 Tratamientos con anticuerpos hormonales: Trastuzumab (Herceptin)	7
2.2.3.3 Tratamiento con Quimioterapia	7
2.2.3.4 Terapia Combinada	8
2.2.4 Uso de líneas celulares en la investigación en Cáncer de Seno	8
2.2.5 Contribuciones de la citogenética en la investigación en Cáncer de Seno	9
2.2.6 Inestabilidad Cromosómica y Heterogeneidad Clonal	10
3. ESTADO DE ARTE	10
4. PLANTEAMIENTO DEL PROBLEMA	13
4.1 Pregunta de Investigación	13
5. OBJETIVOS	14
5.1 Objetivo General	14
5.2 Objetivos Específicos	14
6. METODOLOGÍA	14
6.1 Líneas Celulares	14
6.2 Tratamientos en las Líneas Celulares	15
6.3 Proliferación Celular	16
6.4 Preparación de los extendidos cromosómicos y núcleos interfásicos	16
6.5 Evaluación de la Inestabilidad Cromosómica y heterogeneidad clonal en las líneas celulares usando hibridación in situ por fluorescencia (FISH)	16
6.6 Análisis Estadísticos	17
7. PRODUCTOS	17
8. IMPACTO	18
REFERENCIAS	18
CAPITULO II. THE ROLE OF CHROMOSOMAL INSTABILITY IN	26

CANCER AND THERAPEUTIC RESPONSES	
Abstract	27
1. Introduction	27
2. CIN and Cancer	27
2.1 Mechanisms of CIN	29
2.2 The role of CIN in cancer Development and progression	30
2.2.1 Breast Cancer (BC)	31
2.2.2 Prostate Cancer (PC)	32
2.2.3 Colorectal Cancer (CRC)	33
2.2.4 Cervical Cancer (CC)	34
2.2.5 Endometrial Cancer (EC)	34
2.2.6 Bladder Cancer (BCA)	35
2.2.7 Multiple Myeloma (MM)	36
2.2.8 High Hyperdiploid Actue Lymphoblastic Leukemia (HeH ALL)	36
3. The Role of CIN in Anticancer Therapy	37
3.1 Therapeutic Strategies Based on CIN	37
3.2 The Association between CIN and Poor Prognoses	38
3.3 CIN and its Potential Beneficial Effects for Therapy	39
4. CIN in Naturally Occurring Congenital Aneuploidy of Non-cancerous Origin	39
5. Conclusions	40
References	40
 CAPITULO III. ROLE OF CHROMOSOMAL INSTABILITY AND CLONAL HETEROGENEITY IN THE THERAPY RESPONSE OF BREAST CANCER CELL LINES	51
Abstract	52
1. Introduction	52
2. Results	53
2.1 Definition of CIN levels and CH in control cell lines	53
2.2 Variation of CIN and CH in HER2 – cells after	54
2.2.1 MCF7 cells	54
2.2.2 ZR751 cells	55
2.2.3 MDA-.MB468 cells	57
2.3 Variation of CIN and CH in HER2+ cells	58
2.3.1 BT474 cells	58
2.3.2 KPL4 cells	59
3. Discussion	61
4. Materiales and Methods	64
4.1 Cells Lines	64
4.2 Treatments	65
4.3 Proliferation assay	65
4.4 Metaphase and nuclei spreads	65
4.5 Fluorescence in situ Hybridization (FISH) and CIN evaluation	66
4.6 Data Analysis	66

5. Conclusions	67
References	68
CONCLUSIONES FINALES	72
RECOMENDACIONES	73
ANEXOS	74

LISTA DE TABLAS

	Pág
Tabla 1. Tratamientos aplicados a las líneas celulares de Cáncer de Seno	15
Tabla 2. Chromosomal alterations observed at high frequencies in HeH ALL	37

LISTA DE FIGURAS

	Pág
Figura 1. CIN Characteristics	28
Figura 2. Numerical and structural CIN arise during mitotic chromosome segregation errors	30
Figura 3. Chromosomal alterations most frequently observed in several types of cancer	31
Figura 4. CIN Positive or Negative Response to Treatment.	38
Figura 5. Chromosomal Instability (CIN) and Shannon diversity index (SDI) in untreated breast cancer cell lines	54
Figura 6. Effects of tamoxifen (TAM), docetaxel (DOC), doxorubicin (DOX), TAM+DOC and TAM+DOX treatments for 24 h, 48 h, and 96 h on cell proliferation in (A-B) MCF7 cells, (C-D) ZR751 cells and (E-F) MDA-MB468 cells.	55
Figura 7. Chromosomal Instability (CIN) and Shannon Diversity Index (SDI) (indicative of clonal heterogeneity) in HER2+ breast cancer cells treated with tamoxifen (TAM), docetaxel (DOC), doxorubicin (DOX), TAM+DOC and TAM+DOX at various time points	56
Figura 8. Representative FISH images of the MCF7 breast cancer cells after A) DOC treatment and B) TAM+DOX treatment.	57
Figura 9. Representative FISH images of the MDA-MB468 breast cancer cells after A) TAM+DOC treatment and B) DOX treatment	58
Figura 10. Effects of tamoxifen (TAM), docetaxel (DOC), doxorubicin (DOX), herceptin (HT), TAM+DOC, TAM+DOX, TAM+HT, HT+DOC and HT+DOX treatments for 24 h, 48 h, and 96 h on proliferation in (A-B) BT474 cells and (C-D) KPL4 cells	59
Figura 11. Chromosomal Instability (CIN) and Shannon Diversity Index (SDI) (indicative of clonal heterogeneity) induced by tamoxifen (TAM), docetaxel (DOC), doxorubicin (DOX), Herceptin (HT), TAM+DOC, TAM+DOX, TAM+HT, HT+DOC and HT+DOX in HER2+ breast cancer cells at each treatment time point	60
Figura 12. CIN threshold at under which cells are sensitive or resistant to treatments	64

LISTA DE ANEXOS

	Pág
Anexo 1. Chromosomal Instability (CIN) and Shannon Diversity Index (SDI) for all cell lines before and after treatments at 24 h, 48 h and 96 h.	74
Anexo 2. Chromosomal Instability (CIN) and cell proliferation in MCF7 cells before and after treatments at 24 h, 48 h and 96 h.	79
Anexo 3. Chromosomal Instability (CIN) and cell proliferation in ZR751 cells before and after treatments at 24 h, 48 h and 96 h.	80
Anexo 4. Chromosomal Instability (CIN) and cell proliferation in MDA-MB468 cells before and after treatments at 24 h, 48 h and 96 h.	81
Anexo 5. Chromosomal Instability (CIN) and cell proliferation in BT474 cells before and after treatments at 24 h, 48 h and 96 h.	82
Anexo 6. Chromosomal Instability (CIN) and cell proliferation in BT474 cells before and after treatments at 24 h, 48 h and 96 h.	83
Anexo 7. Figure 1. Representative FISH images of the KPL4 breast cancer cells after A) DOC treatment and B) TAM+DOC treatment.	84





RESÚMEN

Este estudio cubre un área de gran importancia en la investigación del cáncer de seno (CS), relacionada con el estudio de los efectos de la inestabilidad cromosómica (IC) y su asociación con la respuesta a diversos regímenes terapéuticos usados comúnmente en el tratamiento de esta enfermedad. Teniendo en cuenta que el CS es una enfermedad muy heterogénea y que las pacientes responden de manera diferente al tratamiento, la identificación de la relación existente entre los niveles de IC con la respuesta a la terapia podría contribuir al desarrollo de nuevas estrategias terapéuticas con el fin de aumentar las tasas de supervivencia de pacientes con CS y disminuir los efectos secundarios causados por la terapia.

Los resultados de esta investigación se presentan en tres capítulos, en los cuales se muestra cada uno de los aspectos importantes para el estudio, así como los resultados obtenidos en torno a la evaluación de la IC y heterogeneidad clonal en cinco líneas celulares representativas de cuatro subtipos tumorales de CS y su asociación con la respuesta al tratamiento en términos de modificación de la proliferación celular.

El primer capítulo incluye las generalidades relacionadas con la formulación del problema, la justificación, los objetivos y la metodología de la propuesta así como la información necesaria para hacer una correcta introducción al desarrollo de la investigación. El segundo capítulo, describe el papel de la inestabilidad cromosómica en cáncer. La inestabilidad cromosómica, además de su implicación en el diagnóstico y pronóstico del cáncer, puede ser potencialmente explotada como un objetivo terapéutico al desempeñar un papel importante en la respuesta terapéutica. Este capítulo corresponde al primer artículo resultado de esta investigación publicado en la revista internacional indexada *Cancers*. El tercer capítulo, muestra el papel de la inestabilidad cromosómica y la heterogeneidad clonal en la respuesta al tratamiento en las líneas celulares de CS: MCF7 (receptor de estrógenos (RE) positivos (RE+)/receptor de progestágenos (RP) positivo (RP+)/receptor 2 del factor de crecimiento epidérmico humano (HER2) negativo (HER2-), ZR751 (RE+/RP+/HER2-), BT474 (RE-/RP-/HER2+), KPL4 (RE+/RP+/HER2+) y MDA-MB468 (RE-/RP-/HER2-). Los resultados obtenidos sugieren que existe un umbral de IC que, cuando se excede, conduce a citotoxicidad o supervivencia celular, este umbral de IC depende del estado de expresión de RE y HER2. Por lo tanto, una mayor comprensión del papel de la IC y heterogeneidad clonal en la respuesta a la terapia en CS, junto con estudios prospectivos futuros que evalúen la IC en pacientes, podrían contribuir a la optimización de los regímenes terapéuticos existentes y a la disminución de los efectos secundarios en esta neoplasia. Este tercer capítulo corresponde al segundo artículo producto de la investigación, actualmente sometido a la revista *Cancers*.



CAPÍTULO I

GENERALIDADES



1. INTRODUCCIÓN

El cáncer es una enfermedad común y actualmente es considerado un problema de salud pública, siendo una de las principales causas de muerte a nivel mundial. En conjunto, a nivel global cada año los cánceres de pulmón, seno y colorrectal, son los que presentan las mayores tasas tanto de incidencia como de mortalidad (Paolo Vineis, 2014). En Colombia, el cáncer de seno (CS) es el cáncer diagnosticado con mayor frecuencia y la principal causa de muerte por cáncer en mujeres. Esta enfermedad ha mostrado una alta morbilidad y esta comúnmente asociada con una amplia variedad de factores de riesgo tales como predisposición genética, expresión de RE y amplificación/sobre-expresión de HER2, entre otros (Yi-Sheng Sun, 2017).

La decisión terapéutica para el manejo de pacientes con CS se basa no sólo en la evaluación de factores pronósticos, como RE y HER2, sino en la evaluación de parámetros clínicos y patológicos. En particular, el tamaño del tumor, el grado histológico, histotipo y ganglios linfáticos cumplen un papel importante en la planificación de las estrategias terapéuticas (tratamiento hormonal y/o quimioterapia con diferentes agentes). Sin embargo, aunque este ha sido un enfoque exitoso, algunos pacientes recaen y/o eventualmente desarrollan resistencia a los tratamientos (Huang B, 2014; Göran Jönsson, 2010; Berry DA, 2000; Parisot JP, 1999). Por lo tanto, la identificación de mecanismos y marcadores pronósticos y predictivos confiables se constituye en una necesidad en la investigación en CS. Un blanco terapéutico prometedor es la determinación de la inestabilidad cromosómica (IC), una característica común en tumores sólidos (Pikor L, 2013).

La IC, reconocida como un "hallmark del cáncer" (Hanahan D, 2011), que se define como la tasa (variabilidad célula a célula) de ganancia o pérdida de cromosomas completos (IC numérica - Aneuploidia) o fracciones de cromosomas (IC estructural) (Tanaka K, 2016), favorece el crecimiento simultáneo de diversas subpoblaciones tumorales, conduciendo a heterogeneidad genómica inter e intra-tumoral (heterogeneidad clonal) (Tanaka K, 2016; Geigl JB, 2008; Gagos S, 2005). Es importante resaltar que tanto la IC como la heterogeneidad clonal han sido ampliamente asociadas con progresión del cáncer, aumentada invasividad y respuesta a la terapia (Gagos S, 2005; Thomson AB, 2002; Heng HH1, 2013; Chandrakasan S, 2011).

La IC y la heterogeneidad clonal conducen a interacciones reguladoras de genes y concentraciones variables de proteínas, que podrían afectar las respuestas celulares a los tratamientos farmacológicos (Jasbani, 2015) al inducir una sensibilidad variable al fármaco. De hecho, investigaciones recientes sugieren relaciones paradójicas entre la IC extrema y un mejor resultado en pacientes con diversos tipos de cáncer (Birkbak NJ, 2011; Jamal-Hanjani M, 2015), en donde la expresión de RE es indicativa de la buena o mala respuesta terapéutica en pacientes con CS (Roylance R, 2011). Estas observaciones muestran la complejidad asociada con la selección de una terapia apropiada para diferentes tipos de cáncer cuyo objetivo es la IC, y sugieren que puede existir un umbral de IC que, cuando se excede, podría inducir citotoxicidad celular o supervivencia celular. Desafortunadamente, estos umbrales de IC son teóricos y podrían ser específicos para cada tipo de cáncer.

Considerando que los parámetros y/o marcadores clinicopatológicos, inmunohistoquímicos y moleculares actuales continúan dejando un número significativo de pacientes en riesgo de sobre o bajo tratamiento, y más aún, en riesgo



de recaer y/o eventualmente desarrollar resistencia a la terapia, es fundamental determinar y estandarizar nuevos marcadores o herramientas pronósticos y predictivos de fácil acceso. Una mayor comprensión de las relaciones existentes entre los niveles de IC con la respuesta a la terapia (reducción en la proliferación celular), podría permitir comprender no sólo el papel que desempeña la IC en la respuesta a la terapia, sino que junto con estudios prospectivos futuros en pacientes con CS, podría contribuir al desarrollo de nuevas estrategias terapéuticas personalizadas y además permitir predecir el beneficio de la terapia hormonal y la quimioterapia y/o respaldar nuevas estrategias terapéuticas.

Teniendo en cuenta lo anterior nosotros (i) evaluamos la IC y la heterogeneidad clonal en cinco líneas celulares representativas de cuatro subtipos moleculares de CS que expresan diferencialmente RE y HER2 y (ii) establecimos su asociación con la respuesta a diversos regímenes terapéuticos en términos de modificación de la proliferación celular. En esta investigación, observamos que la IC intermedia está relacionada con la sensibilidad a los medicamentos de acuerdo con tres características, que incluyen el estado RE y HER2, el nivel preexistente de IC de las células tumorales y la IC inducida por los tratamientos. Además, se observó que todas las terapias utilizadas en nuestro estudio (monoterapia o terapia combinada) promovieron la IC, lo que influyó en la respuesta a la terapia.

2. MARCO CONCEPTUAL

2.1 Generalidades del Cáncer

El cáncer es un grupo de enfermedades complejas conocidas por producir alteraciones en los procesos celulares tales como proliferación, diferenciación y muerte celular (Erenpreisa J, 2007). Esta pérdida generalizada de control se atribuye a la acumulación gradual de mutaciones en los genes implicados en la regulación de estos procesos (proto-oncogenes, genes supresores de tumores y genes reparadores del ADN), que a su vez conducen a la transformación celular marcada por la división celular incontrolada, la evasión del sistema inmune, la invasión y la metástasis (Erenpreisa J, 2007).

Según la Agencia Internacional para la Investigación del Cáncer (IARC), en particular el programa GLOBOCAN, se estima que en el 2018 hubo 18.1 millones de nuevos casos de cáncer y 9,6 millones de muertes relacionadas con esta enfermedad (Bray F, 2018), frente a 14.1 y 8.2 millones respectivamente en 2012 (Torre, 2015), y se prevé que el número de nuevos casos aumente a cerca de 23,6 millones para el 2030 (Forouzanfar, 2016).

En Colombia, se presentaron aproximadamente 101.893 nuevos casos de cáncer y alrededor de 46.057 muertes en el 2018, siendo el cáncer de próstata (26.6%) y el cáncer de seno (24.8%) los más frecuentes en hombres y mujeres respectivamente (Bray F, 2018; Pardo C, 2017). Tanto la incidencia como la mortalidad muestran un aumento en comparación con años anteriores, que puede explicarse por el crecimiento y envejecimiento de la población mundial, la adopción de estilos de vida asociados con cáncer, como el tabaquismo, la inactividad física y los malos hábitos alimenticios (Torre, 2015), así como la variación en la distribución de los factores de riesgo ligados fuertemente al desarrollo económico y social.

2.2 Cáncer de seno



2.2.1 Epidemiología

El CS es el cáncer diagnosticado con mayor frecuencia y la principal causa de muerte por cáncer en mujeres, representando el 11.6% (2.088.849 millones) del total de nuevos casos de cáncer y el 6,6% (626.679) del total de muertes por cáncer en 2018 a nivel mundial (Bray F, 2018). La IARC estimó que para el 2018 en Colombia, se presentaron 13.380 (14.73%) nuevos casos de CS y aproximadamente 3.702 (8.89%) muertes atribuidas a esta enfermedad, convirtiéndose así en la primera causa de muerte en mujeres en nuestro país (Bray F, 2018). Aunque los factores hereditarios y genéticos representan del 5% al 10% de los casos de CS, los factores que contribuyen a la variación internacional de las tasas de incidencia van desde factores reproductivos y hormonales hasta factores relacionados con la nutrición y la cultura (Torre, 2015).

2.2.2 Clasificación del Cáncer de Seno

El CS generalmente se clasifica por su aspecto histológico, dependiendo de las células de las que derive el tumor, en CS no invasivo y CS invasivo. El CS no invasivo (*in situ*) se caracteriza porque las células cancerígenas no se extienden a los tejidos externos, a los lóbulos o conductos, por ejemplo: Carcinoma lobular *in situ* (CLIS) y carcinoma ductal *in situ*. Por otra parte, el CS invasivo se presenta cuando las células anormales de los lóbulos o los conductos lácteos se dividen en las proximidades del tejido mamario (Harris LN, 2016) como el carcinoma lobular infiltrante (ILC), el carcinoma ductal infiltrante y el carcinoma medular y carcinoma mucinoso (Akram M, 2017).

La aparición de técnicas moleculares (microarrays) ha permitido la determinación de perfiles de expresión génica, la identificación de la heterogeneidad tumoral y la clasificación molecular del CS. De esta manera, se han propuesto cinco subtipos basados en la expresión de receptores de RE, receptores de progesterona (RP), genes asociados a estrógenos, genes asociados con la inducción de proliferación, tales como el gen *HER2* y otros genes localizados en la región del amplicón *HER2* en el cromosoma 17 (Reis-Filho JS, 2011). Estos subtipos son:

-]) *Luminal A* (RE+, RP+/-, HER2-) y *Luminal B* (RE+, RP+/-, HER2+)

Estos subtipos tienen un buen pronóstico y se caracterizan por expresar genes asociados al RE, tales como *LIV1* y *ciclina D1*, así como queratinas de bajo peso molecular (CK7, CK8, CK18, etc). La expresión del RE es una condición para el tratamiento con Tamoxifen (TAM) o inhibidores de aromatasa. Sin embargo, este subtipo tumoral muestra una pobre respuesta a la quimioterapia neoadyuvante. Estos carcinomas se asocian a características morfológicas favorables tales como tamaño tumoral pequeño (menos de 2 cm), tumores diferenciados de grado histológico bajo a moderado, ganglios linfáticos axilares negativos y una etapa temprana en el momento del diagnóstico. Ambos subtipos expresan altos niveles de BCL-2 y bajos índices de proliferación cuando (Ki-67) (Reis-Filho JS, 2011).

-]) *HER2-positivo* (RE-, RP-, HER2+)

El CS positivo para HER2 (HER2 +) presenta expresión aumentada de los genes ubicados en la misma región cromosómica donde se encuentra *HER2* (cromosoma 17q), incluyendo el receptor del factor de crecimiento ligado a la proteína 7 (GRB7) y se asocia a menudo a otros marcadores de mal pronóstico. Otros genes alterados en



este subtipo tumoral son: topoisomerase II-alfa (*TOP2A*), GATA4 y algunos genes implicados en angiogénesis y en proteólisis (Reis-Filho JS, 2011). Este subtipo es resistente a terapia endocrina y generalmente se trata con trastuzumab.

]) *Basal (RE-, RP-, HER2- y CK5/6, CK14, p63 y/o EGFR+)*

Este subtipo se caracteriza por la sobreexpresión de genes de las citoqueratinas de la capa basal (CK5/6, CK17) y genes relacionados con la proliferación celular. A menudo, estos tumores presentan mutaciones en el gen *TP53*, sobreexpresan el receptor del factor de crecimiento epidérmico (EGFR) y muestran ausencia de expresión de RE, HER2 y otros genes relacionados. Este subtipo se asocia con mutaciones en el gen *BRCA1*. Adicionalmente, muestra el comportamiento más agresivo entre todos los subtipos tumorales de CS, a pesar de su alta sensibilidad a la quimioterapia (Reis-Filho JS, 2011).

]) *Similar a la mama normal (RE-, RP-, HER2-, CK5/6-, CK14-, p63- y/o EGFR-)*

Este carcinoma tiene características similares al tejido mamario normal, muestra fuerte expresión de genes específicos del tejido adiposo y baja expresión de genes del epitelio luminal (Kim J, 2012). Recientemente, un nuevo subtipo clasificado como "bajo en Claudina" ha sido identificado (Göran Jönsson, 2010).

2.2.3 Tratamientos contra el Cáncer de Seno

A medida que se adquieren más conocimientos sobre la fisiopatología y el comportamiento genético y genómico de la enfermedad, el tratamiento para pacientes con CS ha ido cambiando según los avances tecnológicos, los consensos médicos y la evolución de los pacientes frente a los medicamentos (Jia Y, 2017). El CS es una enfermedad heterogénea en la que cada paciente tiene características individuales, lo que ha llevado a la búsqueda de nuevos marcadores con el fin de mejorar no sólo el diagnóstico sino también el pronóstico y obtener una mejor respuesta a la terapia (Peralta, 2011). Actualmente, las estrategias para el tratamiento del CS están dirigidas a atacar marcadores específicos que están funcionalmente alterados (Peralta, 2011), tales estrategias incluyen tratamiento hormonal, tratamientos con anticuerpos monoclonales, quimioterapia y terapias combinadas.

2.2.3.1 Tratamiento hormonal

El manejo terapéutico de los pacientes positivos para los RE (RE+) consiste en la aplicación de estrategias endocrinas que buscan bloquear los RE con el agente anti-estrógeno Tamoxifen (TAM) o eliminar la cantidad de ligando (estrógenos), ya sea suprimiendo las gónadas en mujeres premenopáusicas (ovarectomía) o mediante el uso de inhibidores de la aromatasa, en mujeres posmenopáusicas. Estas estrategias se utilizan tanto en casos tempranos como metastásicos; sin embargo, no todos los pacientes responden a la terapia endocrina, y aquellos que inicialmente responden, con el tiempo adquieren resistencia a la terapia (Berry DA, 2000; Parisot JP, 1999). Del mismo modo, en pacientes con amplificación del gen HER2 y sobreexpresión de la proteína, el tratamiento consiste en utilizar anticuerpos monoclonales que reconocen el dominio extracelular del gen HER2 (Trastuzumab), así como inhibidores del dominio tirosina-quinasa del receptor HER2 (tal como lapatinib), o incluso silenciando el gen con ARN de interferencia (ARNi) (Tai W, 2010).



J Tamoxifen

El TAM es un modulador selectivo de receptores de estrógenos (SERM), debido a que su efecto parece estar restringido a tumores RE+. El uso de este agente antiestrógeno (dosis de 20 mg/día) ha demostrado: reducir la incidencia de CS en un 38% en mujeres sanas con alto riesgo de adquirir la enfermedad, disminuir la probabilidad de recurrencia en CS temprano, prevenir el desarrollo de cáncer en el seno opuesto y reducir el riesgo de desarrollar CS invasivo en mujeres que han tenido carcinoma ductal *in situ* (DCIS). Todo lo anterior se explica por la capacidad que tiene el TAM de reducir la proliferación celular y de inducir apoptosis en las células malignas de mama, entre otros (Mandlekar S, 2001; Salami S, 2003).

En CS metastásico, ha sido indicado que más del 50% de pacientes con tumores RE+ tratados con TAM logran detener la progresión tumoral; sin embargo, casi todos los pacientes con enfermedad metastásica y el 40% de los pacientes que reciben TAM como terapia adyuvante, eventualmente recaen y mueren por causa de esta enfermedad. Aún más, mujeres posmenopáusicas con CS en las primeras etapas, que inicialmente respondieron bien a TAM, puede llegar a adquirir resistente a la droga y desarrollar tumores recurrentes (tumores de seno y endometrio) (Pearce ST, 2004).

Además, Mizutani y colaboradores (Mizutani A, 2004) reportaron que el TAM es carcinogénico y que tiene un alto potencial mutagénico, bloqueando la replicación y causando rupturas cromosómico, así como translocaciones y delecciones que contribuyen a la iniciación y/o progresión tumoral. Adicionalmente, investigaciones posteriores demostraron que ratas tratadas con TAM presentaban varias aberraciones cromosómicas numéricas y estructurales (Sargent LM1, 1994) incluyendo: ruptura cromosómica, delecciones, aneuploidía y endorreduplicaciones (Kedia-Mokashi N, 2010). La inducción de anomalías cromosómicas podría deberse a la formación de aductos de ADN favorecidos por TAM, lo que conduce a inestabilidad cromosómica (IC), característica muy común asociada a carcinogénesis. Sin embargo, el tipo y frecuencia de anomalías cromosómicas y los mecanismos por los cuales TAM induce IC son aún desconocidos y su efecto está siendo estudiado para establecer su implicación en la progresión de la enfermedad y en la modificación de la respuesta terapéutica.

2.2.3.2 Tratamientos con anticuerpos hormonales: *Trastuzumab (Herceptin)*

Trastuzumab, también conocido como Herceptin, es un anticuerpo monoclonal humanizado que ha sido altamente efectivo en el tratamiento de mujeres con CS positivas para HER2 (HER2+), en etapas tempranas o avanzadas e incluso estadios metastáticos. Este compuesto se une al dominio extracelular de HER2, interfiere con las vías de señalización intracelular mediadas por este receptor y suprime la proliferación celular. Interesantemente, se ha reportado una mayor tasa de supervivencia cuando este medicamento se usa en combinación con quimioterapia (docetaxel, vinorelbina y componentes de platino) (Barton S, 2011).

2.2.3.3 Tratamiento con Quimioterapia

La quimioterapia está diseñada para inducir apoptosis a tantas células tumorales como sea posible con el uso de dosis máximas toleradas de medicamentos. El mecanismo



principal de los agentes citotóxicos, ya sea directos o indirectos, es causar daño al ADN e interrumpir la replicación del ADN en las células en proliferación (Elisabetta Munzone, 2015). Dentro de los fármacos más usados en quimioterapia en CS incluyen Doxorubicina y Docetaxel.

) Doxorubicina

La doxorrubiciна (DOX), es una clase de antraciclina y uno de los agentes quimioterapéuticos más importantes en el tratamiento del cáncer en general. Esta clase de antraciclina, interfiere con la biosíntesis de ácidos nucleicos al intervenir en la función de la topoisomerasa II, evitando la relajación del ADN superenrollado y bloqueando así la transcripción y replicación del ADN, por lo tanto, ejercen su acción en todas las fases del ciclo celular (Wang S, 2004). La cardotoxicidad es el efecto adverso más importante de la DOX, no se conoce exactamente el mecanismo por el que se produce la cardotoxicidad por antraciclinas, pero parece estar relacionado con la producción de radicales libres por parte de los compuestos hierro-antraciclina (Pérez, 2009).

) Docetaxel

Los taxanos como el docetaxel (DOC) pertenecen a un grupo de medicamentos antineoplásicos utilizados como tratamiento de cáncer localmente avanzado y metastásico, posterior a la no respuesta ante antraciclinas (Hudis Zepeda-Castilla, 2008). La acción de los taxanos se sustenta en la estabilización de la tubulina y la posterior formación de microtúbulos no funcionales que desencadenan una mitosis errónea al intervenir con algunos componentes críticos en la fase S de la división celular (Zhang, 2019). En presencia del DOC se observan importantes daños celulares en los productos de la mitosis, con acumulación de células multinucleadas no viables (Chan, 2019). Estudios recientes en pacientes con CS sugieren que la neuropatía periférica es un efecto secundario del tratamiento con taxanos, causada por la neurotoxicidad de los mismos (Chan, 2019).

2.2.3.4 Terapia combinada

En la actualidad, se están estudiando otras estrategias que combinan trastuzumab con agentes anti-microtúbulos (trastuzumab-emtansina T-DM1), así como el uso de inhibidores de PI3K y mTOR o inhibidores de proteínas de choque térmico (Tanespimycin) (Barton S, 2011). Del mismo modo, en pacientes con CS positivos tanto para HER2 como para RE, se usan tratamientos combinados, incluyendo trastuzumab con letrozol, trastuzumab con anastrazole y lapatinib con letrazole (estos fármacos bloquean ambos receptores). En estos casos, un aumento del 50% en la supervivencia del paciente ha sido reportada (SR, 2010).

2.2.4 Uso de líneas celulares en investigación en CS

En la investigación en CS y en general en cáncer, el uso de líneas celulares se constituye en una herramienta esencial, ya que estas, además de ser estudiadas para dilucidar la biología del CS, han sido ampliamente usadas para el establecimiento de nuevas estrategias terapéuticas (Barretina J, 2012; Chavez KJ, 2010). Dado que las líneas celulares son fácilmente propagadas, y genéticamente manipuladas, amplia información sobre su transcriptoma, genoma, epigenoma, así como sobre su heterogeneidad genómica, ha sido generada (Lacroix M, 2004; Chin K, 2006; Sproul D,



2011; Sacks, 1988).

Actualmente existe una gran variedad de líneas celulares usadas para la investigación en CS, dentro de las cuales se encuentran **MCF7, ZR751, BT474, KPL4 y MDA-MB468** representativas de cuatro subtipos tumorales de CS.

J MCF7

Representativas del subtipo tumoral Luminal A (RE+/HER2-). La línea celular MCF7 fue aislada del tejido de glándula mamaria derivado del sitio metastásico de derrame pleural, perteneciente a una mujer caucásica de 69 años, con adenocarcinoma y morfología epitelial (ATCC, 2018).

J ZR751

La línea celular ZR751 es de tipo epitelial y derivada de una mujer blanca, de 63 años aislada a partir de un derrame ascítico maligno de un carcinoma ductal del seno derecho (Sacks, 1988). Esta línea celular es representativa del subtipo tumoral Luminal A (RE+/HER2-).

J BT474

La línea celular BT474 fue aislada del tejido de glándula mamaria derivado del conducto, es de tipo epitelial y perteneciente a una mujer caucásica, de 60 años (ATCC, 2018). Esta línea celular es representativa del subtipo tumoral Luminal B (RE-/HER2+).

J KPL4

La línea celular KPL4 es de tipo epitelial y derivada de una mujer de 51 años aislada a partir de un derrame preural de un carcinoma inflamatorio del seno (ATCC, 2018). Esta línea celular es representativa del subtipo tumoral HER2+ (RE+/HER2+).

J MDA - MB468

La línea celular MDA - MB468 derivado del sitio metastásico de derrame pleural, perteneciente a una mujer de 51 años de raza étnica afro descendiente, con adenocarcinoma y morfología epitelial (ATCC, 2018). Esta línea celular es representativa del subtipo tumoral Basal o Triple negativo (RE-/HER2-).

2.2.5 Contribuciones de la Citogenética a la investigación en Cáncer de Seno

La citogenética es una herramienta invaluable en el diagnóstico y la investigación en cáncer (Peralta, 2000). La contribución de la citogenética es concluyente para el diagnóstico de cáncer, dado que ha contribuido a: la identificación de aberraciones cromosómicas, mayor comprensión de los procesos de transformación maligna y proporciona información útil para el desarrollo y validación de nuevos tratamientos (Ribeiro, 2019).

Aunque el bandeo cromosómico se utiliza ampliamente para identificar anomalías cromosómicas, la resolución es limitada debido a que muchas anomalías incluyen cambios en pequeños segmentos de cromosomas que no pueden ser detectados por citogenética convencional, por lo que se han desarrollado técnicas como la Hibridación *In Situ* por Fluorescencia (FISH) (Ribeiro, 2019; Carrillo, 2012). FISH combina técnicas citogenéticas con moleculares, y se basa en el uso de sondas de ADN marcadas con



fluorescencia, que reconoce su secuencia complementaria sobre el material genético a evaluar y permite detectar o confirmar anomalías genéticas o cromosómicas (Bartlett, 2010). FISH es una técnica eficiente con alta reproducibilidad para identificar la ubicación de secuencias únicas en los cromosomas metafásicos y facilitar su ubicación en preparaciones bandeadas o no bandeadas (Barton, 2011). Esta técnica permite no sólo una estimación de la heterogeneidad clonal dentro del tumor sino que permite además clasificar y diferenciar los tumores aneuploidos: con una alta (aneuploidía inestable) o baja (aneuploidía estable) heterogeneidad clonal (Popescu Berns, 2007).

2.2.6 Inestabilidad Cromosómica y heterogeneidad clonal

La IC se define como el incremento de la tasa de ganancia o pérdida de cromosomas o fragmentos de cromosomas durante la división celular, generando anomalías a nivel cromosómico. Los tumores con IC se caracterizan por presentar cariotipos con una aneuploidía global, amplificaciones, delecciones, translocaciones, inversiones, entre otras características moleculares (Pikor L, 2013), que permiten el crecimiento de distintas subpoblaciones celulares conduciendo a heterogeneidad celular (Gagos S, 2005).

Estudios recientes en IC le han atribuido posibles implicaciones clínicas, asociándola con buen o mal pronóstico en tumores sólidos, esto como posible consecuencia del aumento de la heterogeneidad (Endesfelder D, 2014; Li R, 2005; Vargas-Rondón N, 2017). De hecho, crece la evidencia de que la IC puede afectar negativamente el progreso del tumor (sobre todo en los subtipos RE-) (Roylance R, 2011; Birkbak NJ, 2011), debido a los cambios cromosómicos numéricos y estructurales que resultan en una acumulación de eventos genómicos perjudiciales para el rendimiento y supervivencia de las células tumorales (Endesfelder D, 2014; Johnston SRD, 2015), por otra parte, los niveles altos de inestabilidad cromosómica pueden ser supresores de tumores debido a la generación de cariotipos inviables (Sansregret L, 2018). Los mecanismos por los que se produce la IC no se encuentran bien descritos, sin embargo, se cree que es el reflejo de disfunciones o daños en el proceso de duplicación y segregación cromosómica en la mitosis (Kwei KA, 2010).

La IC no es estudiada de manera rutinaria en clínica (McGranahan N, 2012), sin embargo con propósitos en investigación, se utilizan variedad de tecnologías para su detección, desde aquellas con enfoques unicelulares, hasta técnicas robustas con enfoques multicelulares, cada una con sus ventajas, alcances a distinto nivel y desventajas; sin embargo, ninguna técnica actual es capaz de medir la tasa de cambio o nivel de variabilidad dentro de una población celular, por lo cual existe la necesidad de la generación de técnicas que sean capaces de medir la IC en el tiempo para poder evaluar verdaderamente su implicación en la respuesta al tratamiento (Pikor L, 2013).

El método más directo y que proporciona una medida más precisa de la inestabilidad cromosómica es la hibridación *in situ* por fluorescencia (FISH), que utiliza sondas específicas para detectar la variación célula a célula del número de copias del cromosoma (Roylance R E. D.-H., 2014).

3. ESTADO DE ARTE

El CS es el cáncer diagnosticado con mayor frecuencia y la principal causa de muerte por cáncer en mujeres, representando el 11,6% (2.088.849 millones) del total de



nuevos casos de cáncer y el 6,6% (626.679) del total de muertes por cáncer en 2018 a nivel mundial (Bray F, 2018). Según las estadísticas de GLOBOCAN para Colombia en el año 2018, se presentaron 13.380 (14.73%) nuevos casos de CS y aproximadamente 3.702 (8.89%) muertes atribuidas a esta enfermedad, convirtiéndose así en la primera causa de muerte en mujeres en nuestro país (Bray F, 2018).

El CS ha sido comúnmente asociado con una amplia variedad de factores de riesgo incluyendo predisposición genética, expresión de RE y amplificación/sobre-expresión del gen HER2, entre otros. Los RE pertenecen a una familia de proteínas nucleares de unión al ADN, las cuales regulan la transcripción de una amplia variedad de genes implicados en el desarrollo y función de órganos reproductores, en la regulación del ciclo celular, en la replicación del ADN, diferenciación, apoptosis, angiogénesis, supervivencia y progresión tumoral (Rondón-Lagos, 2016). Los RE están altamente relacionados en el desarrollo y progresión del CS, por lo que su detección es ampliamente utilizada como marcador de diagnóstico y de pronóstico, se expresan en el 70%-80% de los tumores de seno y su positividad es un predictor de respuesta a terapia endocrina con TAM, donde pacientes con niveles elevados de RE+ muestran una mejor respuesta a esta terapia en comparación con pacientes con una menor expresión (Dayal, 2015). Aunque pacientes RE- usualmente no responden a estos tratamientos, se ha evidenciado que entre el 5% y el 10% de estos se benefician de terapia con TAM (Dayal, 2015; Abe, 1998). Sin embargo, la respuesta a TAM es frecuentemente de limitada duración debido a que los pacientes desarrollan resistencia (Kumar, 2011), siendo este uno de los principales problemas de la terapia endocrina. El manejo terapéutico de los pacientes RE+ consiste en la aplicación de estrategias endocrinas que buscan bloquear los RE con el agente anti-estrógeno TAM o eliminar la cantidad de ligando (estrógenos), ya sea suprimiendo las góndadas en mujeres premenopáusicas (Ooforectomía) o mediante el uso de inhibidores de la aromatasa, en mujeres posmenopáusicas. Estas estrategias se utilizan tanto en casos tempranos como metastásicos (Malhotra, 2010). Sin embargo, no todos los pacientes responden a la terapia endocrina, y aquellos que inicialmente responden, con el tiempo adquieren resistencia a la terapia (Parisot JP, 1999).

Otro marcador importante en el diagnóstico y tratamiento del CS es el gen *HER2* y su proteína (Peralta-Rodríguez Bánkfalvi, 2000). El proto-oncogen humano *HER2* (también llamado ERBB2) está localizado en el brazo largo del cromosoma 17 (17q11q12) y codifica para una proteína de 185 kD (p185). La proteína *HER2* (p185) pertenece a una familia de cuatro receptores de factores de crecimiento con actividad tirosina quinasa (EGFR, HER2, HER3 y HER4) (Malhotra, 2010). Las actividades biológicas de esta familia de receptores incluyen la activación de vías de señalización celular (MAPK, PI3K/AKT y fosfolipasa C) implicadas en la promoción de la proliferación y de la supervivencia celular, no sólo en células normales sino de manera descontrolada en cáncer (Tai W, 2010; Linggi B, 2006). Este gen se encuentra amplificado y/o sobre-expresado en el 15% de pacientes con esta neoplasia (Furrer D, 2016). Estudios clínicos en CS han indicado que la amplificación de este gen se relaciona con recurrencia (Bankfalvi A, 2000), quimiorresistencia (adriamicina, citoxan, metotrexate, 5-fluorouracilo y TAM), corta supervivencia, tumores de alto grado histológico y ganglios axilares positivos (Seshadri R, 1994), todos considerados como indicadores de mal pronóstico. Por lo tanto, la amplificación del gen *HER2* es considerada como un marcador de mal pronóstico y un factor predictivo de respuesta a agentes quimioterapéuticos, anti-estrogénicos y terapia usando anticuerpos específicos para bloquear la función del receptor (Ross JS, 1999). Dentro de estos anticuerpos se encuentra el Trastuzumab (Herceptin), un anticuerpo monoclonal que



se une al dominio extracelular del receptor *HER2*, interfiriendo con las vías de señalización intracelular mediadas por el mismo, y suprimiendo por ende la proliferación celular (CA., 2007; Valabrega G, 2007). Sin embargo, a pesar de los buenos resultados clínicos, una fracción significativa de los pacientes desarrolla resistencia a este tratamiento (Nahta R, 2006; Berns K, 2007; Lu Y, 2001).

La decisión terapéutica para el manejo de pacientes con CS se basa no solo en la evaluación de factores pronósticos, como el RE y *HER2*, sino en la evaluación de parámetros clínicos y patológicos. En particular, el tamaño del tumor, el grado histológico, histotipo y ganglios linfáticos tienen un papel importante en la planificación de las estrategias terapéuticas (tratamiento hormonal y/o quimioterapia con diferentes agentes). Sin embargo, aunque este ha sido un enfoque exitoso, algunos pacientes recaen y/o eventualmente desarrollan resistencia a los tratamientos (Jonsson G, 2010; Berry DA, 2000; Parisot JP, 1999; Pikor L, 2013). Por lo tanto, la identificación de mecanismos y marcadores pronósticos y predictivos confiables, se constituye en una prioridad en la investigación en CS siendo la IC un blanco terapéutico prometedor.

La IC al ser una fuente de variación genética, favorece adaptación tumoral a ambientes poco favorables (Pikor L, 2013), que se caracteriza por la ganancia o pérdida de fragmentos de cromosomas (IC estructural) o cromosomas completos (IC numérica) promueve la heterogeneidad clonal, y por lo tanto ha sido asociada con progresión del cáncer, aumentada invasividad y respuesta a la terapia (Heng 2013; Chandrakasan 2011).

El CS se caracteriza por poseer cariotipos complejos (inestabilidad cromosómica), con múltiples cromosomas implicados tanto en alteraciones de tipo numérico como en alteraciones de tipo estructural. Dentro de las alteraciones cromosómicas más frecuentemente observadas en CS se encuentran alteraciones que afectan los brazos de los cromosomas 1q, 3p y +7, +8, +20 y amplificaciones génicas en las regiones cromosómicas 8p y 17q. Estudios adicionales, usando Hibridación Genómica Comparativa (CGH), han reportado la presencia de delecciones y amplificaciones de grandes segmentos genómicos incluyendo pérdidas en los brazos de los cromosomas 1p, 1q, 3p, 8p, 11q, 13q, 16q, 17p y 17q (Heim & Mitelman, 2015). Estas observaciones citogenéticas y moleculares muestran que los tumores de seno se caracterizan por ser multicloniales, lo que sugiere la existencia de un alto grado de heterogeneidad intratumoral, causada y mantenida por la IC.

En la investigación en CS y en general en cáncer, el uso de líneas celulares se constituye en una herramienta esencial ya que permiten establecer nuevas terapias mucho más eficientes (Barretina J1, 2012; Chavez KJ, 2010). Debido a que las líneas celulares son fácilmente propagadas, y genéticamente manipuladas, y se tiene una amplia información respecto a ellas, con relación a toda su biología (Lacroix M, 2004; Chin K, 2006; Sproul D, 2011). FISH es la mejor técnica para evaluar distintos tipos de alteraciones cromosómicas, y a su vez, evidenciar la alta heterogeneidad intra-celular en núcleos interfásicos al igual que en secciones de tejido tumoral (Geigl JB, 2008), al permitir clasificar y diferenciar los tumores aneuploides con alta o baja heterogeneidad clonal (Lingle Bartlett, 2010; Rondon-Lagos M, 2014; Chin K1, 2004).

La IC y la heterogeneidad clonal conducen a interacciones regulatorias génicas y concentraciones variables de proteínas, los cuales podrían afectar la respuesta celular a los tratamientos farmacológicos (Dayal, 2015). De hecho, ha sido sugerido que las alteraciones cromosómicas en células tumorales individuales inducen sensibilidad



variable a los tratamientos, promoviendo o favoreciendo la supervivencia de una fracción de células tumorales (selección clonal) (Fedorenko IV, 2015). En efecto, publicaciones recientes han mostrado que tumores de seno HER2+, con distintos patrones de complejidad cariotípica (alta IC), responden mejor al tratamiento con antraciclinas (DOX y epirubicina) y a terapias a base de platino (carboplatino), mientras que tumores con relativa estabilidad cromosómica (baja IC) responden mejor al tratamiento con taxanos (DOC, paclitaxel) (O'Malley FP, 2009; Pikor L, 2013; Bartlett JM1, 2010; Burrell RA1, 2010). Sin embargo, información acerca del papel de la IC en otros subtipos de CS es escasa o ausente.

Teniendo en cuenta la alta frecuencia de pacientes que no responden a la terapia o que con el tiempo desarrollan resistencia a la misma, determinar y estandarizar nuevos marcadores/herramientas pronósticos y predictivos de fácil acceso se convierte en una necesidad en la investigación en CS. Una mayor comprensión de las relaciones existentes entre los niveles de IC con la respuesta a la terapia (reducción en la proliferación celular), podría permitir comprender no sólo el papel que desempeña la IC en la respuesta a la terapia, sino que junto con estudios prospectivos futuros en pacientes con CS, podría contribuir al desarrollo de nuevas estrategias terapéuticas personalizadas y además permitir predecir el beneficio de la terapia hormonal y la quimioterapia y/o respaldar nuevas estrategias para mejorar los resultados en cáncer.

4. PLANTEAMIENTO DEL PROBLEMA

Los avances en investigación epidemiológica y clínica de los últimos años en CS, han permitido identificar una amplia variedad de factores de riesgo genéticos, ambientales, así como también los relacionados con la expresión de RE y amplificación/sobre-expresión del receptor HER2, los cuales aumentan las probabilidades de presentar esta enfermedad. Además de la evaluación de factores pronósticos (RE y HER2), el tamaño del tumor, el grado histológico, histotipo y ganglios linfáticos cumplen un papel importante en la planificación de las estrategias terapéuticas para el manejo de pacientes con esta enfermedad. Sin embargo, aunque este ha sido un enfoque exitoso, algunos pacientes recaen y/o eventualmente desarrollan resistencia a los tratamientos (Huang B, 2014; Jonsson G, 2010; Berry DA, 2000; Parisot JP, 1999), siendo este uno de los principales problemas clínicos significantes en el tratamiento de pacientes con CS.

Teniendo en cuenta que los parámetros y/o marcadores clinicopatológicos, inmunohistoquímicos y moleculares actualmente continúan dejando un número significativo de pacientes en riesgo de recaer y/o eventualmente de desarrollar resistencia a la terapia, es primordial determinar y estandarizar nuevos marcadores o herramientas predictivas confiables que puedan brindar información adicional respecto a la ya existente. Una mayor comprensión del papel que desempeña el nivel de IC y heterogeneidad clonal en la respuesta a la terapia en CS, podría contribuir no sólo a aumentar el conocimiento acerca de los mecanismos implicados en el desarrollo de la resistencia, sino la optimización de los regímenes terapéuticos existentes y la personalización de los mismos, con el fin de aumentar las tasas de supervivencia de pacientes con CS en Colombia, y la disminución de los efectos secundarios.

4.1 Pregunta de investigación



¿Los niveles de inestabilidad cromosómica y heterogeneidad clonal están asociados con el aumento en la proliferación celular y la respuesta a diversos fármacos en las líneas celulares MCF7, ZR751, BT474, KPL4 y MDA-MB468, representativas de los subtipos tumorales de CS Luminal A, Luminal B, HER2+ y Triple Negativo, respectivamente?

5. OBJETIVOS

5.1 Objetivo general

Evaluar inestabilidad cromosómica y heterogeneidad clonal en 5 líneas celulares representativas de cuatro subtipos moleculares de cáncer de seno y establecer su asociación con la respuesta a diversos tratamientos en términos de modificación de la proliferación celular.

5.2 Objetivos específicos

- Ñ Evaluar la IC y HC en líneas celulares de CS expresando diferencialmente RE y HER2.
- Ñ Estratificar las líneas celulares de CS de acuerdo con su nivel de IC y HC.
- Ñ Evaluar la proliferación celular en las líneas celulares control y tratadas con Tamoxifen (TAM), Docetaxel (DOC), Doxorubicina (DOX), Herceptin (HT), y tratamientos combinados (TAM/DOC, TAM/DOX, TAM/HT, HT/DOC y HT/DOX).
- Ñ Establecer en las líneas celulares, asociaciones entre el nivel de IC y HC con la respuesta a los tratamientos, en términos de modificación de la proliferación celular.
- Ñ Establecer correlaciones entre el nivel de IC y HC, con los subtipos de CS (*RE+/HER2-, RE+/HER2+, RE-/HER2+ y RE-/HER2-*)

6. METODOLOGÍA

El tipo de metodología que se aplicó fue de tipo cuantitativo, debido a que los datos obtenidos de cada uno de los métodos y de los instrumentos incluyeron una medición sistemática y un análisis estadístico para su interpretación.

El estudio se realizó en cinco líneas celulares representativas de cuatro subtipos tumorales de cáncer de seno, las cuales expresan diferencialmente *RE* y *HER2*. Las técnicas y metodologías que se aplicaron para recolectar la información comprendieron: Cultivo celular, tratamiento de líneas celulares con diferentes fármacos, evaluación de proliferación celular y FISH.

6.1 Líneas Celulares

Se estudiaron cinco líneas celulares de CS, incluyendo MCF7 (*RE+/RP+/HER2*), ZR751 (*RE+/RP+/HER2*), BT474 (*RE-/RP-/HER2+*), KPL4 (*RE+/RP+/HER2+*) y MDA-MB468 (*RE-/RP-/HER2-*), las cuales expresan diferencialmente *RE* y *HER2*. Estas líneas celulares fueron adquiridas a la ATCC (American Type Culture Collection, Manassas, USA). Las líneas celulares MCF7, ZR75-1, KPL4 y MDA-MB468 fueron



cultivadas en medio RPMI-1640 (Sigma), mientras que la línea celular BT474 se cultivó en medio DMEM (Sigma). Los medios de cultivo se suplementaron con 10% de Suero Fetal Bovino (SFB) (Sigma), 1% penicilina, estreptomicina, fungizona (sigma) y 1% de L-glutamina (2mM) (Invitrogen GmbH, Alemania). Las cinco líneas fueron cultivadas en frascos de cultivo de 75 cm² y posteriormente fueron transferidas a incubadora de CO₂ a 37°C y 95% de humedad. Se realizaron cambios de medio a los cultivos cada 48 horas. Las células se mantuvieron en cultivo hasta alcanzar un 80-90% de confluencia.

6.2 Tratamiento de las líneas celulares

Con el objetivo de evaluar si la IC se correlacionaba con la respuesta al tratamiento, las líneas celulares fueron tratadas con Tamoxifen (TAM), Docetaxel (DOC), Doxorubicina (DOX), Herceptin (HT) y tratamientos combinados entre los anteriores fármacos (TAM/DOC, TAM/DOX, TAM/HT, HT/DOC, HT/DOX). Cada fármaco y/o combinación de estos se adicionó a las líneas celulares de acuerdo con la expresión diferencial de *RE* y de *HER2*. Específicamente, líneas celulares positivas y negativas para el RE fueron tratadas con terapia hormonal (TAM) y combinación de esta con quimioterapia (DOC y DOX), mientras que líneas celulares positivas para HER2 fueron tratadas con HT y combinación de este con quimioterapia (DOC y DOX). La distribución de los tratamientos es indicada en la tabla 1.

Tabla 1. Tratamientos aplicados a las líneas celulares de Cáncer de Seno

Cell line	Receptor status	Individual treatments				Combined treatments				
		TAM	DOC	DOX	HT	TAM+DOX	TAM+DOX	TAM+HT	HT+DOC	HT+DOX
MCF7	ER+/PR+/HER2-	X	X	X		X	X			
ZR75-1	ER+/PR+/HER2-	X	X	X		X	X			
MDA-MB468	ER-/PR-/HER2-	X	X	X		X	X			
BT474	ER+/PR+/HER2+	X	X	X	X	X	X	X	X	X
KPL4	ER-/PR-/HER2+	X	X	X	X	X	X	X	X	X

TAM: Tamoxifen; DOC: Docetaxel; DOX: Doxorubicin; HT: Herceptin

La adición de los diversos tratamientos se realizó una vez los cultivos celulares alcanzaron una confluencia del 80-90%. 48 horas antes de iniciar con los tratamientos, los cultivos celulares fueron lavados con PBS 1X y se realizó cambio de medio a RMPI-1640 (Sigma), libres de rojo fenol y suplementado con 10% de SFB "charcoal-stripped" (Sigma). Lo anterior con el objetivo de remover los esteroides endógenos presentes en el suero y excluir la débil actividad agonista a estrógeno del rojo fenol (Y Berthois, 1986). Transcurridas las 48 horas, los cultivos celulares se incubaron con 0.25% de tripsina-EDTA (Sigma) a 37°C por 3 min.

Posteriormente, la suspensión de células resultante se centrifugó durante 5 min a 1000 rpm. El pellet celular resultante de la centrifugación, se resuspendió en medio completo sin rojo fenol, se depositó en frascos de cultivo y se llevó a incubación a 37°C y 5% de CO₂. Transcurridas 24 horas, las células fueron tratadas con 1 µM TAM (T5648, Sigma) (Sapino A, 1986), 10 nM Docetaxel (01885, Sigma) (Katharina Hartmann, 2012), 0.5 µM Doxorubicina (D1515, Sigma) (Wang S K. E., 2004), 50 µg/ml Herceptin (Roche, Alemania) (Ginestier C1, 2007) y combinaciones de estos (Tabla 1). Las concentraciones a usar fueron estandarizadas en estudios previos, y fueron seleccionadas ya que estas han demostrado ser las más bajas en las que un



efecto en la morfología celular *in vitro* puede ser observada (Sapino A, 1986; Katharina Hartmann, 2012; Wang S K. E., 2004; Ginestier C1, 2007).

Los fármacos fueron previamente disueltos en etanol absoluto y añadidos al medio de cultivo a las concentraciones indicadas anteriormente en tiempos de 24h, 48h y 96h. Células sin tratamiento a 24h, 48h y 96h fueron usadas como controles. Al final de cada tratamiento se midió la proliferación celular y se prepararon los extendidos celulares para posterior evaluación de la IC y la heterogeneidad clonal mediante FISH. Lo anterior se realizó para cada línea celular y para cada tratamiento.

6.3 Proliferación celular

Al final de cada tratamiento, para cada uno de los fármacos y sus respectivas combinaciones (TAM, DOC, DOX, HT, TAM/DOC, TAM/DOX, TAM/HT, HT/DOC Y HT/DOX), se evaluó la proliferación celular mediante el uso del kit ELISA de proliferación celular BrdU (Roche Diagnostics Deutschland GmbH). La medición de la absorbancia (450nm) se llevó a cabo en un lector Bicromático MultiSkan (Labsystems, Midland, Canadá). Cada tratamiento se realizó en 24 repeticiones y los resultados fueron expresados como la media ± desviación estándar (S.D.).

6.4 Preparación de los extendidos cromosómicos y núcleos interfásicos

Una vez los cultivos celulares de cada línea celular, control y tratadas, alcanzaron una confluencia del 80%, fueron incubados con colchicina a 37°C por 2,5 horas. Pasado este tiempo a cada frasco contenido las células, se le adicionó tripsina (1X) a 37°C durante 3 minutos con el fin de despegar las células del frasco. Una vez las células se despegaron en su totalidad, se adicionó medio completo y se realizó suspensión de las mismas. La suspensión celular resultante del paso anterior se depositó en tubos falcón de 15 ml y se centrifugó durante 5 min a 1000 rpm. Al pellet celular resultante se le adicionaron 5 ml de solución hipotónica (0,075 M) a 37°C, durante 12 minutos. Pasado este tiempo se realizó pre-fijación, fijación y lavados con fijador Carnoy frío. La suspensión celular resultante del tratamiento anterior se centrifugó durante 5 min a 1000 rpm. Transcurrido este tiempo, el pellet celular resultante fue usado para la extensión de los preparados cromosómicos en láminas porta-objetos. Así obtenidos, los extendidos cromosómicos fueron posteriormente usados para la evaluación de la IC y heterogeneidad clonal mediante la técnica de FISH.

6.5 Evaluación de la IC y heterogeneidad clonal en las líneas celulares usando *hibridación IN SITU por fluorescencia (FISH)*

La IC fue evaluada sobre los extendidos cromosómicos de las líneas celulares control (sin tratamiento) y tratadas, mediante FISH. Para lo anterior se usaron 6 sondas centrómericas (CEP) para los cromosomas 2, 3, 8, 11, 15 y 17 (todas de Cytocell) y protocolos estandarizados. Los cromosomas 2 y 15 fueron seleccionados considerando que estos cromosomas presentaron alteraciones infrecuentes en el número de copias en una serie de tumores de seno analizados mediante aCGH (Roylance R E. D., 2011). Mientras que los cromosomas 3, 8, 11 y 17 fueron seleccionados considerando que estos cromosomas se encuentran frecuentemente alterados en CS (Heim & Mitelman, 2015). FISH tri-color fue realizado para detectar el número de copias de los cromosomas 2, 8 y 11, y 3, 15 y 17 sobre los extendidos cromosómicos mediante el uso de sondas centroméricas marcadas con fluorocromos de diferentes colores: fluorocromo naranja para CEP2 y CEP3, fluorocromo azul CEP8



y CEP17 y fluorocromo verde para CEP11 y CEP15. Diez áreas seleccionadas al azar de los extendidos celulares fueron adquiridas usando microscopio marca Olympus con el software de citogenética Cytovision System 7.4 (Leica Biosystems Richmond, Inc).

La IC fue evaluada para cada cromosoma en un mínimo de 100 núcleos intactos y separados (sin superposiciones). El nivel de IC para cada línea celular fue definida primero mediante el cálculo del porcentaje de núcleos con un número de señales CEP diferente al número modal (número de cromosomas más común en una población de células tumorales) para cada cromosoma individual, y luego se calculó la media del porcentaje de IC de todos los cromosomas analizados (Lengauer, Kinzler, & Vogelstein, 1997; Munro, 2012). De acuerdo con el nivel de IC (%IC), las líneas celulares fueron clasificadas como: baja IC ($CIN = 0-30\%$), IC intermedia ($CIN = 31\%-70\%$) o IC alta ($CIN > 70\%$). Los cromosomas más variables fueron retenidos y serán utilizados para crear un panel FISH útil para estudios posteriores en pacientes.

6.6 Análisis estadísticos

El nivel de IC observado después de los tratamientos fue comparado con el observado en el control para cada línea celular. La prueba t de Student se realizó para comparar la proliferación celular de líneas celulares tratadas, con líneas celulares no tratadas (control). La heterogeneidad clonal dentro de cada línea celular, se determinó calculando el Índice de Diversidad de Shannon, que integra tanto el número como la abundancia de clones celulares dentro de cada línea celular de acuerdo con métodos previamente publicados (Roylance R.E.D., 2011; Maley C.C, 2006). Análisis descriptivos fueron usados para definir los diferentes niveles de IC y para establecer asociaciones entre tales niveles de IC con la respuesta a los diversos tratamientos, en términos de modificación de la proliferación celular, en las cinco líneas celulares. Todos los análisis estadísticos se llevaron a cabo usando la versión SPSS 21 y valores de $p < 0,05$ fueron considerados como estadísticamente significativos.

7. PRODUCTOS

La difusión de los resultados de esta investigación dentro de la comunidad médica y científica, se realizó mediante la publicación de artículos en revistas indexadas internacionalmente (Q1), así como la participación en congresos nacionales mediante la presentación del trabajo en modalidad oral y poster.

Artículos

- Natalia Vargas-Rondón, Victoria E. Villegas, y Milena Rondón-Lagos. The Role of Chromosomal Instability in Cancer and Therapeutic Responses. *Cancers* (Basel). 2017 Dec 28;10(1). pii: E4. doi: 10.3390/cancers10010004 **(PUBLICADO)**
- Natalia Vargas-Rondón, Erika Pérez-Mora, Victoria E. Villegas, and Milena Rondón-Lagos. Role of Chromosomal Instability and Clonal Heterogeneity in the therapy response in Breast Cancer Cell Lines. *Cancers* (Basel). 2019 **(SOMETIDO)**

Ponencias

- **Nombre del evento:** XII Encuentro Facultad de Ciencias- UPTC **Tipo de evento:** Encuentro **Ámbito:** Nacional Realizado el: 2017-10-04 al 2017-10-06 en TUNJA (BOYACÁ) - Universidad Pedagógica y Tecnológica de Colombia **Productos**



Asociados: IMPLICACIONES DE LA INESTABILIDAD CROMOSÓMICA Y DE LA HETEROGENEIDAD CLONAL EN LA RESPUESTA A LA TERAPIA EN CÁNCER DE SENO **Instituciones asociadas:** Universidad Pedagógica y Tecnológica De Colombia - UPTC - Sede Tunja **Participantes:** Natalia Vargas-Rondón, Victoria E. Villegas, y Milena Rondón-Lagos. **Rol en el evento:** Ponente Póster.

- **Nombre del evento:** LIV CONGRESO NACIONAL Y V INTERNACIONAL DE CIENCIAS BIOLÓGICAS **Tipo de evento:** Congreso Ámbito: Nacional Realizado el: 2019-05-20 al 2019-05-24 en MONTERÍA (CORDOBA) - Centro de convenciones. **Productos Asociados:** INESTABILIDAD CROMOSÓMICA EN LINEAS CELULARES DE CANCER DE MAMA LUMINAL B: CORRELACIONES CON LA RESPUESTA A LA TERAPIA. **Instituciones asociadas:** Universidad de Córdoba. **Participantes:** Natalia Vargas-Rondón, Victoria E. Villegas, y Milena Rondón-Lagos. **Rol en el evento:** Ponente Oral.
- **Nombre del evento:** V ENCUENTRO CIENCIA, MUJER Y TECNOLOGÍA 2019 **Tipo de evento:** Encuentro Ámbito: Nacional Realizado el: 2019-10-03 al 2019-10-03 en DUITAMA (BOYACÁ) – Universidad Pedagógica y Tecnológica de Colombia. **Productos Asociados:** IMPLICACIONES DE LA INESTABILIDAD CROMOSÓMICA EN LA RESPUESTA AL TRATAMIENTO EN LÍNEAS CELULARES DE CÁNCER DE SENO REPRESENTATIVAS DE LOS SUBTIPOS TUMORALES LUMINAL A Y TRIPLE NEGATIVO. **Instituciones asociadas:** Universidad Santo Tomas de Colombia - Universidad Pedagógica y Tecnológica de Colombia. **Participantes:** Natalia Vargas-Rondón, Erika Pérez-Mora, Victoria E. Villegas, and Milena Rondón-Lagos. **Rol en el evento:** Ponente Poster.

8. IMPACTO

Los sectores beneficiados con esta investigación serán el sector científico, el sector académico-investigativo y el sector salud. Los resultados obtenidos de la presente investigación pueden aumentar la comprensión de los mecanismos implicados en el desarrollo de resistencia a la terapia, así como el papel que desempeñan la IC y la HC en la respuesta a la terapia. Los resultados aquí obtenidos pueden ser aplicados en estudios futuros que permitan confirmar el papel de la IC y la HC en pacientes con cáncer de seno, con miras a ser usados en el establecimiento de nuevos enfoques terapéuticos, dirigidos a aumentar las tasas de supervivencia y la reducción de efectos secundarios. Considerando lo anterior, los resultados de la presente investigación benefician además a las personas que padecen esta enfermedad, a sus familias y por ende al país, ya que el CS es la primera causa de muerte en mujeres en Colombia.

REFERENCIAS

- Akram M, I. M. (2017). Awareness and current knowledge of breast cancer. *Biol Res*, 50(1), 33. doi: 10.1186/s40659-017-0140-9
- Abe et al, (1998). Tamoxifen for Early Breast Cancer Trialists. *Collaborative Group*, 1451–67. doi: 10.1109/TTHZ.2014.2336540
- ATCC. (20 de Marzo de 2018). Obtenido de ATTC Credible leads to incredible: <https://www.atcc.org/en.aspx>



- Bankfalvi A, S. R.-D. (2000). Comparative methodological analysis of erbB-2/HER-2 gene dosage, chromosomal copy number and protein overexpression in breast carcinoma tissues for diagnostic use. *Histopathology*, 37(5), 411-9.
- Barretina J1, C. G. (2012). The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. *Nature*, 483(7391), 603-7. doi: 10.1038/nature11003
- Bartlett JM1, M. A. (2010). Predictive markers of anthracycline benefit: a prospectively planned analysis of the UK National Epirubicin Adjuvant Trial (NEAT/BR9601). *Lancet Oncol*, 11(3), 266-74. doi: 10.1016/S1470-2045(10)70006-1
- Barton S, S. C. (2011). Recent developments in treatment stratification for metastatic breast cancer. *Drugs*, 71(16), 2099-113. doi: 10.2165/11594480-000000000-00000
- Berns K, H. H. (2007). A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. *Cancer cell*, 12(4), 395-402. doi: 10.1016/j.ccr.2007.08.030
- Berry DA, M. H. (2000). HER-2/neu and p53 expression versus tamoxifen resistance in estrogen receptor-positive, node-positive breast cancer. *Journal of Clinical Oncology*, 18(20), 3471-9.
- Birkbak NJ, E. A. (2011). Paradoxical relationship between chromosomal instability and survival outcome in cancer. *Cancer research*, 71(10), 3447-52. doi: 10.1158/0008-5472
- Bray F, F. J. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*, 68(6), 394-424. doi: 10.3322/caac.21492
- Burrell RA1, J. N.-F. (2010). Targeting chromosomal instability and tumour heterogeneity in HER2-positive breast cancer. *J Cell Biochem*, 111(4), 782-90. doi: 10.1002/jcb.22781
- CA., H. (2007). Trastuzumab--mechanism of action and use in clinical practice. *N Engl J Med*, 357(1), 39-51.
- Carrillo Barretina, J. C. (2012). The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. *Nature*, 483(7391), 603-607. doi: 10.1038/nature11003
- Chan, Y.-N. J.-W.-J.-Y.-W.-J. (2019). Taxane-Induced Peripheral Neuropathy: Objective and Subjective Comparison Between Paclitaxel and Docetaxel in Patients With Breast Cancer. *Clinical Journal of Oncology Nursing*, 23(5), 494–501. doi: 10.1188/19.cjon.494-501
- Chandrakanan S1, Y. C. (2011). Malignant fibrous histiocytoma two years after autologous stem cell transplant for Hodgkin lymphoma: evidence for genomic instability. *Pediatr Blood Cancer*, 56(7), 1143-5. doi: 10.1002/pbc.22929



- Chavez KJ, G. S. (2010). Triple negative breast cancer cell lines: one tool in the search for better treatment of triple negative breast cancer. *Breast Dis*, 32(2), 35-48. doi: 10.3233/BD-2010-0307
- Chin K, D. S. (2006). Genomic and transcriptional aberrations linked to breast cancer pathophysiology. *Cancer cell*, 10(6), 529-34.
- Chin K, d. S. (2004). In situ analyses of genome instability in breast cancer. *Nat Genet*, 36(9), 984-8. doi:doi: 10.1038/ng1409
- Collaborators, G. 2. (2016). Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet*, 388(10053), 1659-1724. doi: 10.1016/S0140-6736(16)31679-8
- Dayal, J. A. (2015). Quantitation of multiclonality in control and drug-treated tumour populations using high-throughput analysis of karyotypic heterogeneity. *Convergent Science Physical Oncology*, 1(025001), 50-62. doi: 10.1088/2057-1739/1/2/025001
- Elisabetta Munzone, M. C. (2015). Clinical overview of metronomic chemotherapy. *Nature Reviews Clinical Oncology*, 12(11), 631-644. doi: 10.1038/nrclinonc.2015.131
- Endesfelder D, B. R. (2014). Chromosomal instability selects gene copy-number variants encoding core regulators of proliferation in ER+ Breast cancer. *Cancer Res*, 74(17), 4853–63.
- Erenpreisa J, C. M. (2007). Cancer: a matter of life cycle? *Cell Biol Int*, 31(12), 1507-10. doi:doi: 10.1016/j.cellbi.2007.08.013
- Fedorenko IV, W. J. (2015). BRAF Inhibition Generates a Host-Tumor Niche that Mediates Therapeutic Escape. *J Invest Dermatol*, 135(12), 3115-3124. doi: 10.1038/jid.2015.329
- Furrer D, J. S. (2016). Tissue Microarray Is a Reliable Tool for the Evaluation of HER2 Amplification in Breast Cancer. *Anticancer Res*, 36(9), 4661-6.
- Gagos S, I.-F. I. (2005). Chromosome instability in neoplasia: chaotic roots to continuous growth. *Int J Biochem Cell Biol*, 37(5), 1014-33.
- Geigl JB, O. A. (2008). Defining 'chromosomal instability'. *Trends Genet*, 24(2), 64-9. doi: 10.1016/j.tig.2007.11.006
- Ginestier C1, A. J.-J. (2007). ERBB2 phosphorylation and trastuzumab sensitivity of breast cancer cell lines. *Oncogene*, 26(50), 7163-9. doi: 10.1038/sj.onc.1210528
- Göran Jönsson, J. S.-C. (2010). Genomic subtypes of breast cancer identified by. *Breast cancer research*, 12(3). doi:doi: 10.1186 / bcr2596
- Hanahan D, W. R. (2011). Hallmarks of cancer: the next generation. *CellPress*, 144(5), 646-74. doi: 10.1016/j.cell.2011.02.013



- Harris LN, I. N.-A. (2016). Use of Biomarkers to Guide Decisions on Adjuvant Systemic Therapy for Women With Early-Stage Invasive Breast Cancer: American Society of Clinical Oncology Clinical Practice Guideline. *J Clin Oncol*, 34(10), 1134-50. doi: 10.1200/JCO.2015.65.2289
- Heim, S., & Mitelman, F. (2015). *Cancer Cytogenetics: Chromosomal and Molecular Genetic Aberrations of Tumor Cells* (Fourth ed.; Heim, S., Felix., M ed.). Oslo, Norway: Wiley Blackwell.
- Heng Barton, S. y. (2011). Recent Developments in Treatment Stratification for Metastatic Breast Cancer . *Springer*, 71(16), 2100–13. doi: 10.1021/om00028a068
- Heng HH1, B. S. (2013). Chromosomal instability (CIN): what it is and why it is crucial to cancer evolution. *Cancer Metastasis Rev.*, 32(2), 325-40. doi: 10.1007/s10555-013-9427-7
- Huang B, O. Y. (2014). Differential expression of estrogen receptor , 1, and 2 in lobular and ductal breast cancer. *Proceedings of the National Academy of Sciences of the United States of America.*, 111(5), 1933-8. doi: 10.1073/pnas.1323719111
- Hudis Zepeda-Castilla, E. J.-M.-H.-V.-M. (2008). Clasificación molecular del cáncer de mama. *Cir Ciruj*, 76, 87-93. doi: 10.4206/cuad.cir.2011.v25n1-10
- Ilda Patrícia Ribeiro, J. B. (2019). Cytogenetics and Cytogenomics Evaluation in Cancer. *International Journal of Molecular Sciences*, 20(19). doi: 10.3390/ijms20194711
- Jamal-Hanjani M, A. R. (2015). Extreme chromosomal instability forecasts improved outcome in ER-negative breast cancer: a prospective validation cohort study from the TACT trial. *Annals of oncology*, 26(7), 1340-6. doi: 10.1093/annonc/mdv178
- Jasbani H S Dayal, L. A. (2015). Quantitation of multiclonality in control and drug-treated tumour populations using high-throughput analysis of karyotypic heterogeneity. *Convergent Science Physical Oncology*. doi:10.1088/2057-1739/1/2/025001
- Jia Y, C. Y. (2017). Exosome: emerging biomarker in breast cancer. *Oncotarget*, 8(25), 41717-41733. doi: 10.18632/oncotarget.16684
- Johnston SRD, A. R.-H. (2015). Extreme chromosomal instability forecasts improved outcome in ER-negative breast cancer: a prospective validation cohort study from the TACT trial. *Ann Oncol*, 1340-6.
- Jonsson G, S. J.-C. (2010). Genomic subtypes of breast cancer identified by array-comparative genomic hybridization display distinct molecular and clinical characteristics. *Breast cancer research*, 12(3), R42.
- Katharina Hartmann, M. B.-P. (2012). A study of Docetaxel-induced effects in MCF-7 cells by means of Raman microspectroscopy. *Anal Bioanal Chem*, 403(3), 745–753. doi: 10.1007/s00216-012-5887-9



- Kedia-Mokashi N, M. A. (2010). Chromosomal aberration in the post-implantation embryos sired by tamoxifen treated male rats. *Mutat Res*, 703(2), 169-73. doi: 10.1016/j.mrgentox.2010.08.016
- Kim J, L. S. (2012). Comparison between screen-detected and symptomatic breast cancers according to molecular subtypes. *Breast Cancer Res Treat*, 131(2), 527-40. doi: 10.1007/s10549-011-1836-0
- Kumar, R. Z. (2011). The Dynamic Structure of the Estrogen Receptor. *Journal of Amino Acids*, 1-7. doi: 10.4061/2011/812540
- Kwei KA, K. Y. (2010). Genomic instability in breast cancer : Pathogenesis and clinical implications. *Mol Oncol*, 4, 255-66. doi: 10.1016/j.molonc.2010.04.001
- Lacroix M, L. G. (2004). Relevance of breast cancer cell lines as models for breast tumours: an update. *Breast Cancer Res Treat*, 83(3), 249-89.
- Lengauer, C., Kinzler, K., & Vogelstein, B. (1997). Genetic instability in colorectal cancers. *Nature*, 386, 623-627. doi: 10.1038/386623a0
- Li R, H. R. (2005). Chromosomal alterations cause the high rates and wide ranges of drug resistance in cancer cells. *Cancer Genet Cytogenet*, 163(1), 44-56.
- Lindsey A. Torre, M. F. (2015). Global Cancer Statistics, 2012. *CA CANCER J CLIN*, 65(2), 65-87. doi: 10.3322/caac.21262
- Linggi B, C. G. (2006). ErbB receptors: new insights on mechanisms and biology. *Trends Cell Biol*, 16(12), 649-56.
- Lingle Bartlett, J. M. (2010). Predictive markers of anthracycline benefit: a prospectively planned analysis of the UK National Epirubicin Adjuvant Trial (NEAT/BR9601). *The Lancet Oncology*, 11(3), 266–274. doi: 10.1016/S1470-2045(10)70006-1
- Lu Y, Z. X. (2001). Insulin-like growth factor-I receptor signaling and resistance to trastuzumab (Herceptin). *J Natl Cancer Inst*, 93(24), 1852-7. doi: 10.1093/jnci/93.24.1852.
- Maley C.C, G. P. (2006). Genetic clonal diversity predicts progression to esophageal adenocarcinoma. *Nature genetics*, 38, 468-473. doi:10.1038/ng1768
- Malhotra, G. K. (2010). Histological, molecular and functional subtypes of breast cancers. *Cancer Biology and Therapy*, 10(10), 955–960. doi: 10.4161/cbt.10.10.13879
- Mandlekar S, K. A. (2001). Mechanisms of tamoxifen-induced apoptosis. *Apoptosis*, 6(6), 469-77.
- McGranahan N, B. R. (2012). Cancer chromosomal instability: Therapeutic and diagnostic challenges. *Nature Publishing Group*, 13(6), 528-38. doi:10.1038/embor.2012.61



- Mizutani A1, O. T. (2004). Extensive chromosomal breaks are induced by tamoxifen and estrogen in DNA repair-deficient cells. *Cancer Res*, 64(9), 3144-7.
- Munro, A. T. (2012). Chromosome instability and benefit from adjuvant anthracyclines in breast cancer. *Br J Cancer*, 107, 71-74. doi:10.1038/bjc.2012.232
- Nahta R, Y. D. (2006). Mechanisms of disease: understanding resistance to HER2-targeted therapy in human breast cancer. *Nat Clin Pract Oncol*, 3(5), 269-80. doi: 10.1038 / ncponc0509
- O'Malley FP, C. S. (2009). Topoisomerase II alpha and responsiveness of breast cancer to adjuvant chemotherapy. *J Natl Cancer Inst*, 101(9), 644-50.
- Paolo Vineis, C. P. (Febrero de 2014). Global cancer patterns: causes and prevention. *The Lancet*, 383(9916). doi:10.1016 / S0140-6736 (13) 62224-2
- Pardo C, d. V. (2017). Atlas de mortalidad por cáncer en Colombia. Cuarta edición. Bogotá D. C. *Instituto Nacional de Cancerología*, 1, 124.
- Parisot JP, H. X. (1999). Altered expression of the IGF-1 receptor in a tamoxifen-resistant human breast cancer cell line. *Br J Cancer*, 79(5), 693-700.
- Pearce ST, J. V. (2004). The biological role of estrogen receptors alpha and beta in cancer. *Crit Rev Oncol Hematol*, 20(1), 3-22.
- Peralta, M. O. (2011). Cáncer de mama: estrategias de prevención y vigilancia según nivel de riesgo. *Revista Médica Clínica Las Condes*, 22(4), 436-443. doi: 10.1016/S0716-8640(11)70448-5
- Peralta-Rodríguez Bánfalvi, À. S.-D. (2000). Comparative methodological analysis of erbB-2/HER-2 gene dosage, chromosomal copy number and protein overexpression in breast carcinoma tissues for diagnostic use. *Histopathology*, 37(5), 411–419. doi: 10.1046/j.1365-2559.2000.00984.x
- Pérez, C. A. (2009). Cardiotoxicidad tardía inducida por antraciclinas . *Medicina Clínica*, 133(8), 311–313. doi:10.1016/j.medcli.2009.04.003
- Pikor L, T. K. (2013). The detection and implication of genome instability in cancer. *Cancer Metastasis Rev*, 32(3), 341-52. doi: 10.1007/s10555-013-9429-5
- Popescu Berns, K. H. (2007). A Functional Genetic Approach Identifies the PI3K Pathway as a Major Determinant of Trastuzumab Resistance in Breast Cancer. *Cancer Cell*, 12(4), 395–402. doi: 10.1016/j.ccr.2007.08.030
- Reis-Filho JS, P. L. (2011). Gene expression profiling in breast cancer: classification, prognostication, and prediction. *Lancet*, 378(9805), 1812-23. doi: 10.1016/S0140-6736(11)61539-0
- Rondon-Lagos M, V. D.-G. (2014). Differences and homologies of chromosomal alterations within and between breast cancer cell lines: a clustering analysis. *Molecular cytogenetics*, 7(1), 8. doi: 10.1186 / 1755-8166-7-8



- Rondón-Lagos, M. V. (2016). Tamoxifen resistance: Emerging molecular targets. *International Journal of Molecular Sciences*, 17(8), 1-31. doi: 10.3390/ijms17081357
- Ross JS, F. J. (1999). HER-2/neu (c-erb-B2) gene and protein in breast cancer. *Am J Clin Pathol*, 112(1), 53-67.
- Roylance R, E. D. (2011). Relationship of extreme chromosomal instability with long-term survival in a retrospective analysis of primary breast cancer. *Cancer Epidemiol Biomarkers Prev*, 20(10), 2183-94. doi: 10.1158/1055-9965.EPI-11-0343
- Roylance R, E. D.-H. (2014). Expression of regulators of mitotic fidelity are associated with intercellular heterogeneity and chromosomal instability in primary breast cancer. *Breast Cancer Res Treat*, 148(1), 221-9.
- Sacks, P. G.-P. (1988). Establishment and characterization of three new continuous cell lines derived from human breast carcinomas. *Cancer Research*, 48(10), 2858-66.
- Salami S, K.-T. F. (2003). Biochemical studies of apoptosis induced by tamoxifen in estrogen receptor positive and negative breast cancer cell lines. *Clin Biochem*, 36(4), 247-53.
- Sansregret L, V. B. (2018). Determinants and clinical implications of chromosomal instability in cancer. *Nat Rev Clin Oncol*, 15(3), 139–50.
- Sapino A, P. F. (1986). Estrogen- and tamoxifen-induced rearrangement of cytoskeletal and adhesion structures in breast cancer MCF-7 cells. *Cancer Res*, 46(5), 2526-31.
- Sargent LM1, D. Y. (1994). Tamoxifen induces hepatic aneuploidy and mitotic spindle disruption after a single in vivo administration to female Sprague-Dawley rats. *Cancer Res*.
- Seshadri R, H. D. (1994). The relative prognostic significance of total cathepsin D and HER-2/neu oncogene amplification in breast cancer. *Int J Cancer*, 56(1), 61-5.
- Sproul D, N. C. (2011). Transcriptionally repressed genes become aberrantly methylated and distinguish tumors of different lineages in breast cancer. *Proceedings of the National Academy of Sciences of the United States of America*, 108(11), 4364-9.
- SR, J. (2010). New strategies in estrogen receptor-positive breast cancer. *Clin Cancer Res*, 16(7), 1979-87. doi: 10.1158/1078-0432.CCR-09-1823
- Tai W, M. R. (2010). The role of HER2 in cancer therapy and targeted drug delivery. *J Control Release*, 146(3), 264-75. doi: 10.1016/j.jconrel.2010.04.009
- Tanaka K, H. T. (2016). Chromosomal instability: A common feature and a therapeutic target of cancer. *Biochim Biophys Acta*, 1866(1), 64-75. doi: 10.1016/j.bbcan.2016.06.002



- Thomson AB, W. W. (2002). Treatment of paediatric Hodgkin's disease. a balance of risks. *Eur J Cancer*, 38(4), 468-77.
- Valabrega G, M. F. (2007). Trastuzumab: mecanismo de acción, resistencia y perspectivas futuras en el cáncer de mama que sobreexpresa HER2. *Ann Oncol*, 18(6), 977-84. doi: 10.1093 / annonc / mdl475
- Vargas-Rondón N, V. V.-L. (2017). The Role of Chromosomal Instability in Cancer and Therapeutic Responses. *Cancers (Basel)*, 10(1), E4. doi: 10.3390/cancers10010004
- Wang S, K. E. (2004). Doxorubicin induces apoptosis in normal and tumor cells via distinctly different mechanisms. intermediacy of H₂O₂- and p53-dependent pathways. *J Biol Chem*, 279(24), 25535-43. doi: 10.1074/jbc.M400944200
- Wang S, K. E. (2004). Doxorubicin induces apoptosis in normal and tumor cells via distinctly different mechanisms. intermediacy of H₂O₂- and p53-dependent pathways. *J Biol Chem*, 279(24), 25535-43. doi: 10.1074/jbc.M400944200
- Y Berthois, J. A. (1986). Phenol red in tissue culture media is a weak estrogen: implications concerning the study of estrogen-responsive cells in culture. *Proc Natl Acad Sci U S A*, 83(8), 2496–2500. doi: 10.1073/pnas.83.8.2496
- Yi-Sheng Sun, Z. Z.-N.-J.-Y. (2017). Risk Factors and Preventions of Breast Cancer. *International Journal of Biological Sciences*, 13(11). doi: 10.7150/ijbs.21635
- Zhang, X. L. (2019). Trastuzumab-Coated Nanoparticles Loaded With Docetaxel for Breast Cancer Therapy . *Dose-Response*, 17(3), 155932581987258. doi:10.1177/1559325819872583



CAPÍTULO II

THE ROLE OF CHROMOSOMAL INSTABILITY IN CANCER AND THERAPEUTIC RESPONSES

Publicado en: Cancers (Basel). 2017 Dec 28; 10(1). pii: E4. doi:
[10.3390/cancers10010004](https://doi.org/10.3390/cancers10010004)



Abstract: Cancer is one of the leading causes of death, and despite increased research in recent years, control of advanced-stage disease and optimal therapeutic responses remain elusive. Recent technological improvements have increased our understanding of human cancer as a heterogeneous disease. For instance, four hallmarks of cancer have recently been included, which in addition to being involved in cancer development, could be involved in therapeutic responses and resistance. One of these hallmarks is chromosome instability (CIN), a source of genetic variation in either altered chromosome number or structure. CIN has become a hot topic in recent years, not only for its implications in cancer diagnostics and prognostics, but also for its role in therapeutic responses. Chromosomal alterations are mainly used to determine genetic heterogeneity in tumors, but CIN could also reveal treatment efficacy, as many therapies are based on increasing CIN, which causes aberrant cells to undergo apoptosis. However, it should be noted that contradictory findings on the implications of CIN for the therapeutic response have been reported, with some studies associating high CIN with a better therapeutic response and others associating it with therapeutic resistance. Considering these observations, it is necessary to increase our understanding of the role CIN plays not only in tumor development, but also in therapeutic responses. This review focuses on recent studies that suggest possible mechanisms and consequences of CIN in different disease types, with a primary focus on cancer outcomes and therapeutic responses.

Keywords: Chromosomal Instability; Therapeutic resistance; Cancer outcomes; Cancer prognosis; Predictive markers

1. Introduction

Cancer is a multifactorial disease, which is characterized by the presence of a population of cells with complex and heterogeneous karyotypes [1]. Therapeutic decisions for cancer patients are primarily based on clinical and pathological parameters. In particular, tumor size, histological grade, histotype and immunohistochemical results of prognostic factors play major roles in planning therapeutic strategies [2] (e.g. targeted therapy or chemotherapy). Although this has been a successful approach, many patients relapse and/or eventually develop resistance. Despite the fact that vast technological improvements have increased our understanding of human cancers as heterogeneous diseases, current clinicopathological, immunohistochemical and molecular parameters/markers leave significant numbers of patients at risk for over- or under-treatment.

A promising therapeutic target for cancer is chromosome instability (CIN), a common feature of solid tumors. CIN has been recognized as a source of genetic variation, favoring tumor adaptations to stressful environments and cytotoxic anticancer drugs [3]. In cancer research, both numerical (aneuploidy) and structural CIN have been shown to impact carcinogenesis and possibly therapeutic responses; however, although CIN has been associated with cancer therapy, contradictory findings have been reported regarding its implications for the therapeutic response [4-6]. Thus, it is necessary to increase our understanding of the role CIN plays not only in tumor development but also in responses to therapy. In this review, we will discuss the impact of CIN on the prognosis of many disease types, including cancer.

2. CIN and Cancer



CIN, defined as a defect that involves loss or rearrangement of the chromosomes during cell division [4], has been recognized as hallmark of cancer [7] and a source of genetic variation that favors tumor adaptations to stressful environments and cytotoxic anticancer drugs. CIN is a common feature of solid tumors and can be classified as numerical CIN or structural CIN [8]. Numerical CIN is characterized by gain or loss of whole chromosomes (aneuploidy) [3], while structural CIN is characterized by gain or loss of fractions of chromosomes [3] (Figure 1).

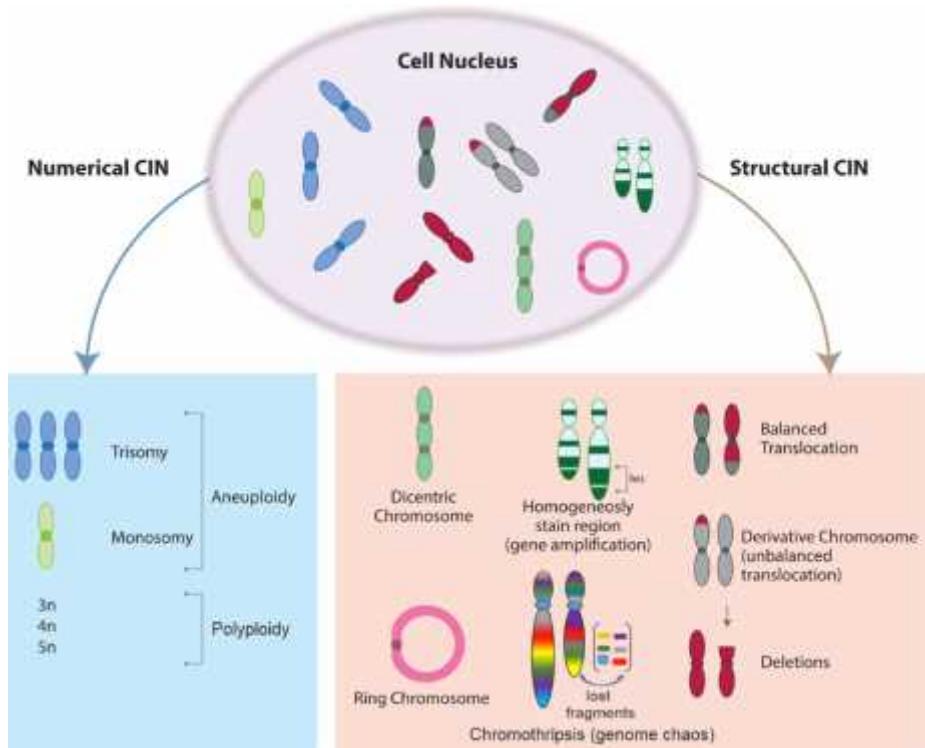


Figure 1. CIN-Characteristics. CIN is characterized by aberrant distribution of chromosomes to the daughter cells deviating from the modal number (*numerical CIN – aneuploidy and euploidy*) or an elevated frequency of structural chromosome aberrations such as gain or loss of partial chromosomes (*structural CIN*).

Aneuploidy refers to the state of abnormal chromosome numbers, which can be stable or unstable. Unstable aneuploidy may favor the simultaneous growth of various tumor subpopulations leading to inter and intratumoral genomic heterogeneity [3,9,10].

In addition, in cancers with elevated numerical and structural CIN, genome chaos has been also observed. Genome chaos defined as a process of complex, rapid genome re-organization, is characterized by the presence of extreme structural and numerical chromosomal alterations [11]. Many of these chromosomal alterations are non-recurrent abnormalities (NCCAs) and since these changes are not clonal (clonal chromosomal alterations - CCAs), they are widely ignored and therefore not reported [12].

CCAs are defined as chromosomal alterations observed at least twice within 20 to 40 randomly examined mitotic figures (range of occurrence greater than 30%) [13]. NCCAs are defined as non-recurrent chromosomal alterations observed at a frequency of less than 4% among 50–100 mitotic figures [13] and are characteristic of chaotic



genomes. Examples of NCCAs include deletions, translocations, gene amplifications, inversions, chromothripsis, chromoplexy, dicentric chromosomes and duplications, among others [11,14].

Considering that CCAs are indicative of stable karyotypes and NCCAs of unstable karyotypes, it has been suggested that NCCAs are the main indicators of structural CIN and cancer evolution [15]. However, in spite of the above, NCCAs have been widely ignored, since have been considered as insignificant "noise" [16,17] and as *in vitro* culture artifact. Therefore, information about the presence of these alterations in many types of cancer is scarce, which limits the possibility of obtaining additional information about genomic diversity and, therefore, intra and inter tumor heterogeneity [12,17].

Taking into account that several studies has suggested that NCCAs are essential in the evolution of cancer [17,18] and therefore can be useful in the establishment of both tumor heterogeneity and CIN [13,17], their inclusion in the study of cancer is urgent, essential and relevant.

2.1. Mechanisms of CIN

The mechanisms underlying CIN remain poorly understood but likely reflect dysfunctional chromosome duplication or segregation during mitosis (Figure 2). Within these mechanisms are: kinetochore-microtubule attachment errors, aberrant sister chromatid cohesion, abnormal centrosome replication, telomere attrition, and the spindle assembly checkpoint (SAC) abnormalities [19], among others. Cancer cells with CIN mis-segregate a chromosome approximately once every one to five divisions, compared with a rate of one chromosome per hundred cell divisions in stable, diploid cell lines [20,21].

During mitosis and meiosis, the spindle assembly checkpoint (SAC) acts to maintain genome stability by delaying cell division until accurate chromosome segregation can be guaranteed [22], which ensures that anaphase is triggered only after all kinetochores are bound to spindle microtubules [23]. In order for chromosome segregation to be carried out with high fidelity, prior to the start of anaphase, the kinetochores must capture the microtubules of the spindle and connect the sister chromatids of each chromosome to the poles of the opposite spindle (amphitely fixation). Once all chromosomes achieve proper bi-oriented attachments to spindle microtubules (amphitely fixation), the SAC is inactivated, and chromosome segregation and cell division to proceed. If the chromosomes are not correctly attached to the spindle (erroneous attachments), kinetochores activate the SAC network, which inhibits the initiation of anaphase and preserves the cohesion of the sister chromatid [22,24,25]. Erroneous attachments include cases where only one kinetochore is attached to a spindle pole (monotely), both sister kinetochores are attached to the same pole (syntely), or one sister kinetochore is attached to both poles (merotely).

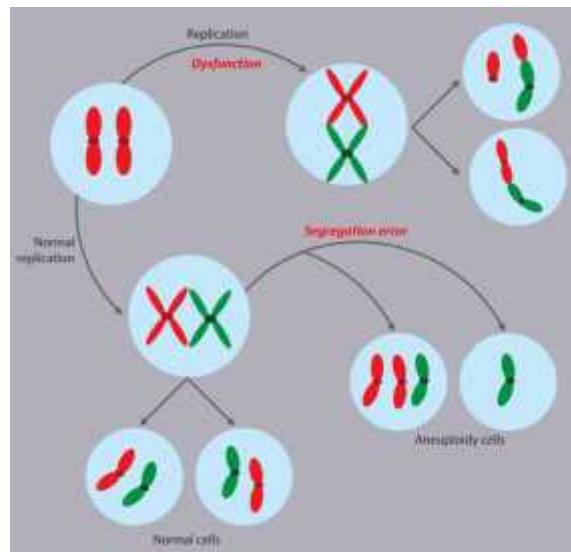


Figure 2. Numerical and structural CIN arise during mitotic chromosome segregation errors. Dysfunctional chromosome duplication or segregation during mitosis can conduct to whole chromosome gains and losses (numerical CIN) and/or alterations in the structure of chromosomes (structural CIN) including translocations, deletions, and derivative chromosome, among other. Both numerical and structural alterations predispose chromosomes to subsequent chromosomal alterations, thereby increasing CIN.

Further, merotelic attachments are characterized by the absence of tension between sister kinetochores and are not detected by the SAC, and without correction, may result in chromosome mis-segregation due to slow chromatid migration speed [26,27]. Merotely is the primary mechanism of CIN in cancer cells [28]. In fact, it has been suggested that uncorrected merotelic attachments are the driving force behind the CIN phenotype observed in approximately 85% of all sporadic carcinomas [29].

2.2. The Role of CIN in Cancer Development and Progression

The role of CIN in the development of cancer is widely debated, since while some researchers consider that CIN is an early event in carcinogenesis that leads to the loss or inactivation of tumor suppressor genes [30-32], others postulate that CIN is a side effect of tumor growth, during which neoplastic cells lose and/or gain chromosomes relatively frequently [33]. In addition, has been indicated that CIN facilitates the acquisition of mutations conferring aggressive or drug-resistant phenotypes during cancer evolution [34].

The impact of aneuploidy on gene expression implies that chromosomal copy number variation leads to an altered stoichiometry of proteins that interact physically or functionally. Stoichiometric perturbations of the protein interaction networks involved in chromosome segregation or the spindle assembly checkpoint can lead to errors in chromosomal segregation, aneuploidy and subsequent CIN [35].

In general, the chromosomal alterations that underlie CIN have emerged as prognostic markers for hematologic cancers and some solid tumors. In addition, the molecular characterization of cytogenetic alterations has provided important information on the mechanisms underlying tumorigenesis and on the treatments that target specific genetic abnormalities. Additionally, both CIN and heterogeneity have been associated with cancer progression, increased invasiveness, poor prognosis and, drug resistance [36-40], this is why some studies have given a clinical value to CIN in



human cancers [39-41]. Furthermore, it has been reported that CIN is highest in the most aggressive and metastatic cancer types [42].

Considering that information regarding NCCAs is scarcely reported, here we indicate the CCAs most frequently observed in several types of cancer (these with higher incidence in the world population), and discuss their relationship with disease development and progression (Figure 3).

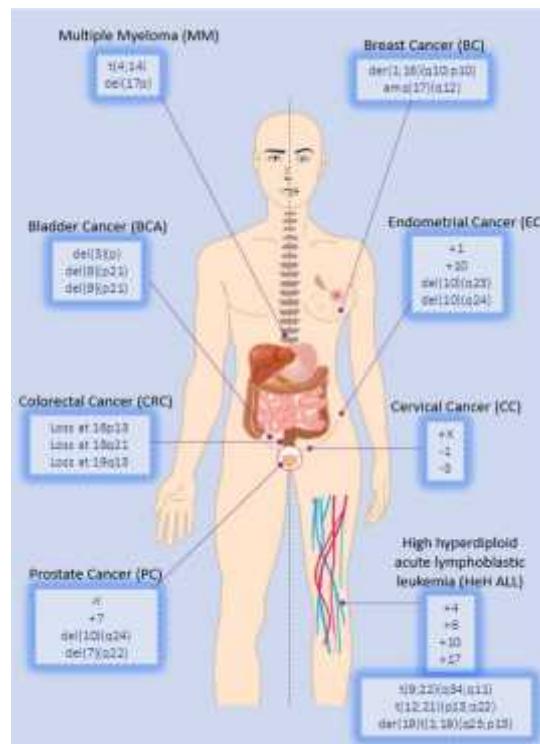


Figure 3. Chromosomal alterations most frequently observed in several types of cancer.

2.2.1. Breast Cancer (BC)

BC is the second most common cancer in the world and the most frequent cancer among women, with an estimated 1.67 million new cases diagnosed in 2012 (25% of all cancers) [43]. BC is a heterogeneous disease, with appreciable patterns of chromosomal alterations. Kwei *et al* (2010) [44] performed genomic profiling and postulated three different patterns of chromosomal alterations, which are differentiated by the frequency and complexity of such alterations. These patterns were called "simple", "amplifier" and "complex". The "simple" pattern is characterized by the presence of few gains or losses of whole chromosome arms. The "amplifying" pattern is characterized by the presence of focal high-level DNA amplifications, and the last pattern, the "complex", is characterized by the presence of copy-number transitions and by numerous low-amplitude changes.

The "simple" pattern, exhibits few copy number alterations, with greater frequency of gains or losses of whole chromosome arms, most characteristically gain of 1q and 16p and loss of 16q. Additionally, a translocation resulting in a derivative chromosome der(1;16)(q10;p10), considered an early event in BC, has also been observed [45-47]. This pattern, also termed "simplex", or "1q/16", is primarily associated with Estrogen Receptor (ER)-positive, moderate to highly differentiated tumors, with luminal A gene-



expression patterns and rarely observed in basal-like and HER2-related tumors [48,49]. Additionally, these alterations are observed in both early and invasive tumors. For instance, gain of 1q and loss of 16q are common in invasive carcinomas [50]. Loss of the long arm of chromosome 16 (16q) is also found in invasive ductal carcinomas, premalignant lesions [51] and in more than 60% of invasive lobular carcinomas. Although *CDH1* (E-cadherin) resides on 16q, to date, there is no evidence to show that loss of 16q in BC leads to the inactivation of this gene[44].

The "Amplifier" pattern is characterized by focal high-level DNA amplifications clustered on one or more chromosome arms. This pattern is associated with the luminal B and HER2-enriched subtypes [48,50]. Frequently amplified sites include 8p12 (*FGFR1*), 8q24 (*MYC*), 11q13 (*CCND1*), 12q15 (*MDM2*), 17q12 (*HER2*) and 20q13 (*ZNF217*). Some of these alterations have been noted to occur together [52], suggesting cooperating events and implying molecular subgroups. Amplified genes play important roles in signaling, cell proliferation, cell-cycle regulation and nucleic acid metabolism [50].

The third class of chromosomal alterations is characterized by a "complex" pattern of many gains and losses of low amplitude, which encompass short chromosomal regions. This pattern, called "complex", results in a segmented profile with many variations of copy numbers, being more common in basal-like and triple-negative BC tumors. In spite of the complex patterns, the most frequent gains are observed in 10p, and the losses in 3p, 4p, 4q, 5q, 14q, 15q, and in some studies 17q [44].

Additionally, elevated aneuploidy is correlated with higher tumor grade, poorer survival and shorter times to recurrence and metastasis in most BC subtypes [53]. These observations suggest that CIN and the resultant alterations have important clinical implications that could be used not only to discriminate between different BC subtypes but also to direct therapy decisions. One of the most notable and classic examples is amplification of *HER2*, which occurs in approximately 15% of BC patients. This gene (also called *ERBB2*) is located on long arm of chromosome 17 (17q12) and encodes a transmembrane tyrosine kinase receptor, whose overexpression is a pharmacological target for the recombinant monoclonal antibody Trastuzumab (herceptin). In addition, the use of combination therapies, which include trastuzumab and chemotherapy, has been reported to reduce the rate of BC death in both the adjuvant and metastatic settings [54].

2.2.2 Prostate Cancer (PC)

PC is the second most common cancer in men. Worldwide, an estimated 1.1 million men were diagnosed with PC in 2012, accounting for 15% of all cancer diagnoses in men, with almost 70% of the cases (759,000) occurring in more developed countries [43].

There is a substantial body of literature that establishes the presence of CIN in PC, and various candidate chromosomes have been suggested to play a role in malignant development, including chromosomes 1, 7, 8, 10, 17 and X [55-57]. For instance, Al-Maghribi *et al.* analyzed numerical CIN (aneuploidy) in PC patients and showed that gain of chromosome 8 was the most frequent change, followed by gain of chromosome 7 and chromosome Y aneusomy [58]. In addition, a strong correlation between chromosomal alterations and prognosis has been also established in PC. For example, tumors with 8q gains or more than two genetic copy number changes are correlated with poor outcomes. In fact, the prognostic significance of 8q gain in PC was recently reported [56].



Additionally, studies of peripheral blood lymphocytes (PBLs) from PC patients have also been performed. These studies found that the X chromosome had a significantly higher mean level of spontaneous breaks in patients compared to those observed in controls. These results showed that spontaneous CIN in PBLs might be a potential biomarker for PC susceptibility [59]. In fact, an increased frequency of CIN in PBLs reflects the early biological effects of genotoxic carcinogens and individual cancer susceptibilities [60].

Furthermore, the metastatic potential of disseminated cell pools from metastatic PC patients was recently investigated. In this study, Holcomb *et al.* detected frequent losses in 8p23, 10q, 13q and 16q, and gains in 8q and Xq, alterations that are frequently identified in PC [61,62]. According to the authors, these results established the basis to elucidate the relationship between genomic alterations of disseminated tumor cells and progressive PC [61].

In addition, Baca *et al.* (2013) [63], by modeling the genesis of genomic rearrangements in PC, identified many DNA translocations and deletions that arise in a highly interdependent manner. Such phenomenon was called by them as "chromoplexy", term used to describe the putative phenomenon of complex genome restructuring. Such complex rearrangement events may disrupting tumor suppressor genes and creating oncogenic fusions in a coordinated way, possibly favoring tumor evolution not only in PC but in other neoplasms. The characterization of Chromoplexia in cancer, which is indicative of structural CIN, could provide information on the initiation and progression of cancer, with broad implications for the detection, prevention and therapy of cancer. Together, these results suggest important implications for CIN in PC development, progression and evolution.

2.2.3. Colorectal Cancer (CRC)

Worldwide, CRC is the third most common cancer in men (746,000 cases, 10.0% of the total) and the second in women (614,000 cases, 9.2% of the total) [43]. CIN has been observed in 65% of CRC cases, lowest in stage 1 and highest in stage 4 disease [42]. Recurrent losses at 16p13 and 19q13, which are significantly associated with bad outcomes in stage 2 and 3 disease, have been observed in CRC [42]. Interestingly both regions co-occurred in the high-risk genetic instability groups. Additionally, allelic loss at chromosome 18q21 has been identified in the 70% of primary colorectal tumors, particularly in advanced-staged disease [64]. Tumor suppressor genes are localized within this region, including the gene *Deleted in Colorectal Carcinoma* (*DCC*), mutations of which are rarely detected in human colorectal tumors (6%) [65,66]. Additionally, *SMAD2* and *SMAD4*, which regulate cell growth, differentiation and apoptosis are also within this region; however, *SMAD2* and *SMAD4* mutations have been found in low frequency in CRCs [67,68].

Further studies found additional chromosomal alterations in CRC. For instance, Shih *et al.* analyzed 32 sporadic colorectal adenomas and identified a relatively high frequency of allelic imbalances on chromosomes 1p (10%), 5q (55%), 8p (19%), 15q (28%), and 18q (28%), with more than 90% of the adenomas showing allelic imbalance of at least one chromosomal arm [69]. In addition, CIN have been also reported in colorectal adenomatous polyposis. For instance, Cardoso *et al.* [70], for studying the aneuploid status of polyps from patients with germline *APC* or *MYH* mutations and found that among 60%–80% of the polyps exhibited aneuploid changes, being the most frequent aberrations the losses of chromosomes 17p, 19q and 22q and the gains of chromosomes 7 and 13 [70]. These findings support the conclusion that chromosomal abnormalities can occur during the early stages of tumorigenesis. In fact, recent reports



have indicated that brain metastases have higher frequency of gains and losses of whole chromosomes and generally more chromosomal aberrations than primary tumors [39,71].

Interestingly, it has also been reported that CIN in CRC can be a therapeutic target. For instance, Swanton *et al* (2007) [72] observed that CIN-positive tumors are intrinsically resistant to taxanes due to the similarities between both: pathways that regulate the separation of chromosomes during mitosis, and pathways involved in taxanes responses. In fact, taxanes function primarily by interfering with spindle microtubule dynamics. When cells are exposed to conditions of prolonged mitotic stress in the presence of microtubule poisonous drugs, like taxanes, the SAC is eventually inactivated and cells can exit mitosis [73].

2.2.4. Cervical Cancer (CC)

CC is the fourth most common cancer in women and the seventh most common overall, with an estimated 528,000 new cases in 2012 [43]. Despite treatment, distant metastasis and nodal recurrence will develop in approximately 30% of CC patients [74]. Numerical and structural chromosomal alterations, or a combination of the two, have been identified during the early stages of CC [75,76]. Structural and numerical chromosome 1 alterations are the most frequent karyotypic change in CC. Among the numerical chromosomal alterations, monosomies and polysomies of chromosomes 1, 3, and X are routinely used as positive genetic biomarkers to diagnose CC and predict the extent of disease progression [77,78]. It is also noteworthy that an increased frequency of spontaneous chromosomal aberrations was observed in patients with precancerous cervical lesions [79], indicating a possible role for CIN in CC progression. In fact, it has been suggested that aneuploidy status is a better prognostic predictor than lymph node status in CC [80].

In addition to the alterations indicated above, micronuclei (MN) have also been observed in CC. MN are extra-nuclear bodies that contain damaged chromosome fragments and/or whole chromosomes that were not incorporated into the nucleus after cell division [81]. MN are therefore the result of CIN. High frequency of MN has been reported in invasive CC, being suggested that the MN score of the exfoliated cervical cells, could be considered as an additional criterion to establish the risk of CC. However, due to the limited number of studies on MN scoring to assess CC risk [82] and on MN scoring in cervical pre-neoplastic and neoplastic conditions [76,83], their implications for CC have not been confirmed. It is noteworthy that according to recent reports, the presence of CIN may help distinguish patients with clinically significant cervical lesions from those who have insignificant lesions, thus discriminating the patient population [84].

2.2.5. Endometrial Cancer (EC)

EC is a disease in which malignant cells form in endometrial tissues and is the leading cause of malignancy in the female genital tract, mostly affecting post-menopausal women [85]. Although the genetic alterations that underlie CIN in EC are poorly understood, a sequential accumulation of genetic alterations from benign to malignant primary lesions has been hypothesized; such alterations include a high frequency of chromosome 10q allelic deletions [86]. In particular, the regions 10q23 [87] and 10q25–q26 [88] have been strongly correlated with EC. Nevertheless, gains of chromosomes 1 and 10 represent the most common cytogenetic abnormality detected in EC [89]. For instance, Muresu *et al.* observed a high frequency of chromosome 1



and 10 trisomy/tetrasomy by analyzing archival tissues from a subset of 86 sporadic EC patients [90].

Interestingly, it has been indicated that CIN can also be found in PBLs from EC patients. The presence of CIN in PBLs is indicative of genome alterations, which are primarily characterized by imperfectly functioning DNA damage repair genes, including genes in the *MMR* family and cell cycle regulators such as *PTEN*, *PIK3*, *KRAS* and *BRAF* [91]. In fact, Bochkov *et al.* reported an increased level of spontaneous chromosomal aberrations in PBLs from EC patients compared with healthy women [92]. Similar results were recently reported by Nesina *et al.*, who indicated that PBLs from most EC patients were characterized by genome destabilization, which was manifested by increased numbers of spontaneous and induced chromosomal damage, hypersensitivity to mutagenic factors, and hidden CIN [93]. Hidden CIN is defined as chromosomal alterations caused by mutagenic exposure to some exogenous or endogenous genotoxic factors [94], which are observed in low frequency (index lower than 1.0). In addition, according to Nessina *et al.*, hidden CIN is one of the manifestations of human genomic instability induced by exposure to radiation, and is a sign of genome destabilization that likely plays a role in EC pathogenesis [35].

2.2.6. Bladder Cancer (BCA)

BCA is one of the most common cancers in the world. An estimated 430,000 BCA cases occurred in 2012, making the disease the ninth most common cause of cancer for both sexes combined [43]. Numerous, nonrandom chromosomal deletions have frequently been detected in BCA [95], including deletions of 3p, 8p, 9p, 11p, 11q and Y. Additionally, gain of 1q, 8q, 17q and 20q have also been found [96-99]. Furthermore, specific chromosomal deletions are associated with BCA progression, and such progression correlates with specific stages of tumor development.

Deletions on chromosome 3p in BCA focused the attention of many researchers, because studies in other types of cancer suggested the presence of tumor suppressor genes in this chromosomal region. For instance, deletions on chromosome 3p have been associated with invasive tumors and have been found in approximately 25% of BCA cases [100,101]. Chromosome 8 deletions, which most frequently affect the region 8p21–22 [102,103], have been observed in 25%–50% of BCA cases [95] and have been significantly correlated with cancer progression [103][55]. Deletions and losses of chromosome 9 have been reported as the most frequently observed in BCA. Loss of chromosome 9 is the only type of chromosome loss identified at the early tumor stages T_0 and T_1 [95], while at later stages loss of other chromosomes, concomitantly with the loss of chromosome 9, were detected [95]. Considering the above, it has been indicated that the total loss of chromosome 9 represents an initial event in the formation of bladder tumors [104]. Deletions of chromosome 9 lead to the loss of genes that encode proteins that activate *Rb* and *p53*, important tumor suppressors [95]. Chromosome 11 deletions are seen in BCA at a high frequency (71.43%) [105], while loss of chromosome 17 has been found in 60% BCA cases and has been associated with advanced disease [95].

The implications of CIN in BCA have reached great importance in recent years, such that clinical tests have been developed specifically to evaluate genomic instability as a molecular marker for the early detection of BCA. In fact, the international consensus panel on bladder tumor markers, recommended a multicolor fluorescence *in situ* hybridization assay to detect copy number variations of chromosomes 3, 7 and 17, and at the 9p21 locus in exfoliated urothelial cells [106]. This test has shown



reasonable performance in detecting BCA in male chemical workers with previous exposure to aromatic amines [107].

2.2.7. Multiple Myeloma (MM)

MM constituted 0.8% of all cancers worldwide (114,000 new cases in 2012) [43]. MM is a cancer formed by malignant plasma cells. Normal plasma cells are found in the bone marrow and are an important part of the immune system. This neoplasia is characterized by the high frequency and consequent accumulation of chromosomal alterations [108]. Further, the complexity of the genomic alterations characteristic of this neoplasm have been correlated with different grades of CIN. Among these alterations, individual abnormalities such as t(4;14) [109] and the deletion of the short arm of chromosome 17 ((del(17p)) [110] are associated with poor outcomes in several treatment contexts [111,112]. Additionally, 17p deletions are also correlated with poor prognosis in MM patients treated with conventional and thalidomide-based chemotherapies[110]. These observations strongly implicated CIN as an important biological and prognostic marker in MM [113].

2.2.8. High Hyperdiploid Acute Lymphoblastic Leukemia (HeH ALL)

In childhood B-cell precursor acute lymphoblastic leukemia (ALL), the most common cytogenetic abnormality is the high hyperdiploidy (51–67 chromosomes), which occurs in 25%–30% of all pediatric B-cell precursor ALL. Of note that high hyperdiploidy has been strongly associated with childhood ALL, since modal numbers of 51-67 have been observed in low frequency in adult B-lineage ALL and rarely in T-cells or in Burkitt's leukemia / lymphoma [114].

HeH ALL is cytogenetically characterized for nonrandom gains of chromosomes X, 4, 6, 10, 14, 17, 18, and 21 [114,115]. Gains of chromosome 21 are the most frequent numerical alterations, showing between three or more copies in 90%–100% of cases [116,117]. In addition to the chromosomal gains, approximately 50% of HeH ALL cases have also structural chromosome aberrations [118,119]. The structural chromosomal alterations observed at high frequencies in HeH ALL are indicated in Table 1 [120][82].

Interestingly, previously published data suggested a cell-to-cell variation in HeH ALL at diagnosis [121,122]; however, further results did not verify this indication. Nevertheless, additional research brought further evidence of a high level of CIN for chromosomes 4, 6, 10, and 17 in HeH ALL patients at initial presentation [123]. In fact, Talamo *et al.* reported that CIN values in HeH ALL patients were higher than those in the negative control group, which would corroborate the potential role of CIN in HeH ALL pathogenesis [123].

Regarding the associations of CIN with outcomes, it has been indicated that whereas trisomies of chromosomes 4 and 6 did not affect prognosis, concurrent trisomies of chromosomes 10 and 17 were associated with better outcomes, and trisomy of chromosome 5 was associated with a poor prognosis [124]. Additionally, Moorman *et al.* found an association between trisomies of chromosomes 4, 10, and 18 and improved outcomes, but only trisomies of chromosomes 4 and 18 had an independent impact in multivariate analysis [119]. Considering these conflicting results, it is important to highlight the need to carry out additional studies to determine whether CIN is a general feature of HeH ALL and to what extent it affects outcomes, as this would be useful information for therapy decisions.



Table 2. Chromosomal alterations observed at high frequencies in HeH ALL

Structural Chromosomal Alterations	Number of Cases
t(9;22)(q34;q11)	991
t(12;21)(p13;q22)	367
der(19)t(1;19)(q23;p13)	263
i(9)(q10)	183
i(17)(q10)	158
i(7)(q10)	155
t(11;19)(q23;p13)	138
del(9)(p21)	134
del(12)(p12)	123
del(11)(q23)	114
del(12)(p13)	77
i(21)(q10)	68
add(19)(p13)	60
dic(9;20)(p11;q11)	52
dic(9;20)(p13;q11)	50

3. The Role of CIN in Anticancer Therapy Responses

The importance of CIN in therapeutic responses results from the fact that chromosomal alterations can lead to altered gene regulatory interactions and varying protein concentrations, both of which could impact cellular responses to treatment [125]. In this regard, it has been indicated that CIN leads to heterogeneous gene expression within a tumor, which could favor the emergence of drug-resistant cell populations, promoting survival in a fraction of tumor cells[126]. However, while some studies have associated high CIN with poor patient outcomes and drug resistance [127], others have indicated that it is associated with better responses [4,128]. In fact, has been indicated that targeting CIN for cancer therapy can induce genome chaos, which contributes to an increased CIN and therefore to the possible acquisition of proliferative advantages and resistance to therapy [129,130].

3.1. Therapeutic Strategies Based on CIN

Before considering CIN as a therapeutic strategy, it must be detected and monitored to know if it can be used as a tool to predict tumor phenotypes, and in this way, contributes to establishing personalized treatment [35,128,131]. This is made possible by determining if CIN makes a tumor more adaptable and better prepared to evolve towards developing resistance to a treatment, or on the contrary, it allows regression of the tumor through cellular collapse [4,34] that leads aberrant cells to undergo apoptosis (Figure 4).

FISH is the most commonly used method to evaluate CIN in patient samples [127,132-134], and studies carried out with this technique in parallel with other findings have shown that increased CIN can positively or negatively impact treatment responses [6,135].

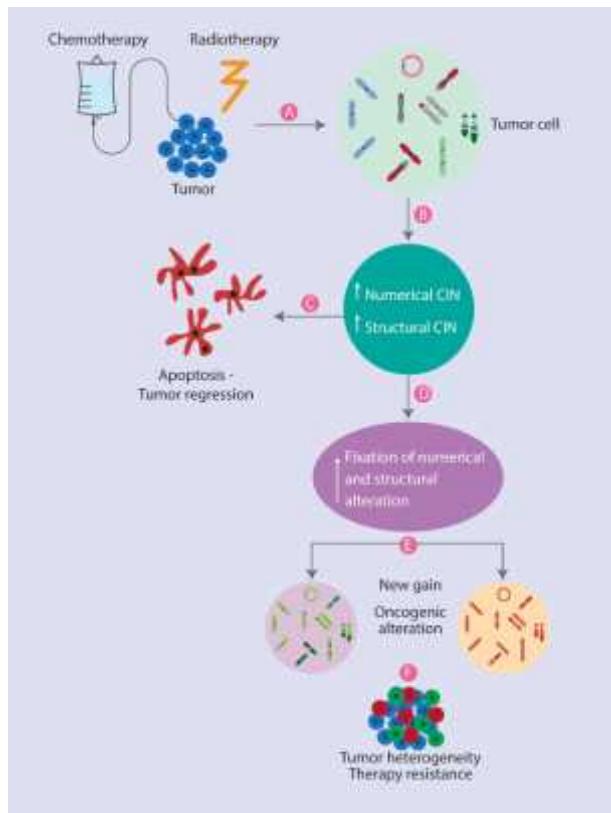


Figure 4. CIN Positive or Negative Response to Treatment. (A) The administration of chemotherapy or radiotherapy to tumor cells (B) can lead to the induction of new numerical and structural chromosomal alterations. This condition could generate two cellular responses: (C) one of them related to the induction of apoptosis (probably due to the excess of genotoxicity), improving the prognosis for the patient by tumor regression, and the other (D) related to the fixation of numerical and structural alterations, (E) consequently leading to clonal expansion of new oncogenic alterations and thus (F) to an overall increase in heterogeneity and development of resistance to therapy.

3.2. The Association between CIN and Poor Prognoses

CIN is generally correlated with tumor development, and innumerable studies have suggested that the aneuploidy that arises as a consequence of CIN in solid tumors favors tumor progression and metastasis [4,5,127,136,137]. It has been demonstrated in several cancer types that CIN-mediated intra-tumoral variability is associated with increased disease aggressiveness, a phenomenon that arises as a consequence of tumor heterogeneity, or the presence of multiple cell clones at the genetic level, which makes the tumor more adaptable and better prepared to evolve resistance [4,5]. For example, studies of ER-positive BC patients [135] and women with ovarian cancer [128] have shown increased CIN in women with resistant disease. In cases where increased CIN contributed to tumor development, therapeutic strategies aimed at decreasing its rate, and thereby inhibiting the processes that led to poor chromosomal segregation or structural changes in cancer cells, have been applied [4].

3.3. CIN and its Potential Beneficial Effects for Therapy



Without a doubt, the role of the CIN in tumor development is a subject that is currently being debated, and contradictory, results generated from animal models show that CIN is poorly tolerated by cancer cells [6].

It has been indicated that although CIN can be beneficial for tumors by providing advantageous alterations, it can also generate vulnerabilities that can be exploited therapeutically. In fact, CIN can generate "synthetic lethal" interactions specifically in tumor cells, by inducing gene dependencies not present in normal cells [138]. For instance, in BC, *BRCA1* and *BRCA2* deficiency leads to a marked sensitivity to poly(ADP-ribose) polymerase (PARP) inhibitors [139,140]. PARP plays an important role in the repair of single-strand breaks, and it is believed that its inhibition leads to the collapse of the replication fork and double-strand breaks, which for their repair depend on homologous recombination. Notably, because *BRCA* carriers are only fully deficient for *BRCA* function in their tumors (not in normal tissues), PARP inhibitors are likely to be highly tumor-specific [44].

Birkbak *et al*, 2011 [6] demonstrated that the extreme CIN in ER negative breast cancer tumors were associated with best prognosis, and similar results have been also observed in ovarian, gastric and non-small cell lung cancer. The therapeutic strategy in cases where CIN generates cell death aims to exacerbate this condition in order to induce tumor cell death [19,33,141-143].

The experimental evidence showing that the increase in CIN in some tumors triggers its reduction has been based mainly on animal models, in which, when treating mice by chemical carcinogens, in order to induce high levels of CIN, culminate in tumor cells death and consequently in reduction or destruction of the tumor, a phenomenon that can be understood as a better prognosis [144,145]. The analysis of these results suggests a possible explanation of how exacerbated CIN could be operating against the tumor: *too much CIN leads to excessive mutations that result in the loss of benefits that the cells had initially acquired toward their tumor transformation* [146,147].

4. CIN in Naturally Occurring Congenital Aneuploidy of Non-Cancerous Origin

CIN plays important roles not only in neoplasia but also in other disease types. Although in humans, whole chromosome aneuploidies are fatal, some of them may be viable but cause congenital diseases: trisomy 13 (Patau syndrome), trisomy 18 (Edwards syndrome), trisomy 21 (Down syndrome), and monosomy X (Turner syndrome). However, it has been reported that the increased rates of CIN observed in these syndromes may increase the risk of developing certain types of cancer. For instance, children with Down syndrome have a high risk of acute myeloid leukemia [148]; Edwards syndrome patients are at risk of developing Wilms' tumor; women with Turner syndrome have an increased risk of gonadoblastoma and childhood brain tumors [149]; men with Klinefelter syndrome have elevated risks of lung cancer, BC and non-Hodgkin's lymphoma [150], while men with Y polysomy have reported rates of cancer incidence similar to those observed in the general population [151]. Interestingly, although few studies have explored CIN in human trisomies, data reported to date, suggest that cells from Down, Edward, Turner and Patau syndrome patients may be karyotypically more unstable than cells from normal diploid individuals.

In addition to above syndromes, CIN has also been observed in Mosaic variegated aneuploidy (MVA) syndrome. This syndrome is a rare disorder in which some cells in the body have an abnormal number of chromosomes instead of the usual 46 chromosomes (aneuploidy). Among the aneuploidies most commonly observed in this syndrome are the monosomies and the trisomies.

MVA syndrome can be caused by mutations in the *BUB1B* gene or the *CEP57* gene. Both genes play very important functions in the process of cell division, since



they encoding proteins involved in mitotic spindle checkpoint and in microtubule stabilization, respectively [152]. MVA syndrome is characterized by multiple mosaic aneuploidies, and a distinct phenotype [153]. Other common characteristic of MVA syndrome is the increased risk of cancer. Cancers that occur most frequently in affected individuals include rhabdomyosarcoma (a cancer of muscle tissue), Wilms tumor (a form of kidney cancer) and leukemia [154,155]. The high incidence of tumors in MVA patients suggests a causal link between CIN and tumor formation [33]. All these results suggested that CIN could contribute to the development of cancer in naturally occurring congenital aneuploidy of non-Cancerous Origin.

5. Conclusions

The tumor-promoting role of CIN has been widely reported in several neoplasms; however, although our understanding of CIN has increased in last years, it is still necessary to consider its consequences in the context of cancer as a heterogeneous and complex disease, instead of one in which CIN only contributes to tumor progression in a simple and autonomous way. In fact, the studies performed to date suggest an important role for CIN in both the outcome and in the responses and resistance to therapy. Thus, CIN is an important target to be considered as we develop novel more effective anticancer treatments. Further, given that cancer is characterized by unstable and chaotic karyotypes, and that such CIN is mainly defined by the presence of NCCAs, identifying and reporting such alterations is clinically relevant. Furthermore, considering that NCCAs are a source of genetic variation not previously recognized, their identification could contribute not only to increase our knowledge about cancer but also to identify new therapeutic opportunities.

Acknowledgments: This work was supported by the Research and Innovation Office of Universidad el Rosario. Graphic designer Elizabeth Cruz Tapias is acknowledged for the illustrations. The authors also thank Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.

Author Contributions: Milena Rondón-Lagos and Victoria E. Villegas, conceived, designed and wrote the manuscript. Natalia Vargas-Rondón wrote sections of the manuscript and made critical revisions. All authors contributed to and approved the final version of the manuscript. The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Nicholson, J.M.; Cimini, D. Cancer karyotypes: Survival of the fittest. *Front Oncol* **2013**, *3*, 148.
2. Soerjomataram, I.; Louwman, M.W.; Ribot, J.G.; Roukema, J.A.; Coebergh, J.W. An overview of prognostic factors for long-term survivors of breast cancer. *Breast Cancer Res Treat* **2008**, *107*, 309-330.
3. Tanaka, K.; Hirota, T. Chromosomal instability: A common feature and a therapeutic target of cancer. *Biochim Biophys Acta* **2016**, *1866*, 64-75.
4. McClelland, S.E. Role of chromosomal instability in cancer progression. *Endocr Relat Cancer* **2017**, *24*, T23-T31.
5. Bakhour, S.F.; Compton, D.A. Chromosomal instability and cancer: A complex relationship with therapeutic potential. *J Clin Invest* **2012**, *122*, 1138-1143.



6. Birkbak, N.J.; Eklund, A.C.; Li, Q.; McClelland, S.E.; Endesfelder, D.; Tan, P.; Tan, I.B.; Richardson, A.L.; Szallasi, Z.; Swanton, C. Paradoxical relationship between chromosomal instability and survival outcome in cancer. *Cancer Res* **2011**, *71*, 3447-3452.
7. Pikor, L.; Thu, K.; Vucic, E.; Lam, W. The detection and implication of genome instability in cancer. *Cancer Metastasis Rev* **2013**, *32*, 341-352.
8. Bayani, J.; Selvarajah, S.; Maire, G.; Vukovic, B.; Al-Romaith, K.; Zielinska, M.; Squire, J.A. Genomic mechanisms and measurement of structural and numerical instability in cancer cells. *Semin Cancer Biol* **2007**, *17*, 5-18.
9. Geigl, J.B.; Obenau, A.C.; Schwarzbraun, T.; Speicher, M.R. Defining 'chromosomal instability'. *Trends Genet* **2008**, *24*, 64-69.
10. Gagos, S.; Irminger-Finger, I. Chromosome instability in neoplasia: Chaotic roots to continuous growth. *Int J Biochem Cell Biol* **2005**, *37*, 1014-1033.
11. Liu, G.; Stevens, J.B.; Horne, S.D.; Abdallah, B.Y.; Ye, K.J.; Bremer, S.W.; Ye, C.J.; Chen, D.J.; Heng, H.H. Genome chaos: Survival strategy during crisis. *Cell Cycle* **2014**, *13*, 528-537.
12. Rangel, N.; Forero-Castro, M.; Rondon-Lagos, M. New insights in the cytogenetic practice: Karyotypic chaos, non-clonal chromosomal alterations and chromosomal instability in human cancer and therapy response. *Genes (Basel)* **2017**, *8*.
13. Heng, H.H.; Regan, S.M.; Liu, G.; Ye, C.J. Why it is crucial to analyze non clonal chromosome aberrations or nccas? *Mol Cytogenet* **2016**, *9*, 15.
14. Burrell, R.A.; McClelland, S.E.; Endesfelder, D.; Groth, P.; Weller, M.C.; Shaikh, N.; Domingo, E.; Kanu, N.; Dewhurst, S.M.; Gronroos, E., et al. Replication stress links structural and numerical cancer chromosomal instability. *Nature* **2013**, *494*, 492-496.
15. Heng, H.H.; Bremer, S.W.; Stevens, J.; Ye, K.J.; Miller, F.; Liu, G.; Ye, C.J. Cancer progression by non-clonal chromosome aberrations. *J Cell Biochem* **2006**, *98*, 1424-1435.
16. Mitelman, F. Recurrent chromosome aberrations in cancer. *Mutat Res* **2000**, *462*, 247-253.
17. Heng, H.H.; Liu, G.; Stevens, J.B.; Abdallah, B.Y.; Horne, S.D.; Ye, K.J.; Bremer, S.W.; Chowdhury, S.K.; Ye, C.J. Karyotype heterogeneity and unclassified chromosomal abnormalities. *Cytogenet Genome Res* **2013**, *139*, 144-157.
18. Dereli-Oz, A.; Versini, G.; Halazonetis, T.D. Studies of genomic copy number changes in human cancers reveal signatures of DNA replication stress. *Mol Oncol* **2011**, *5*, 308-314.
19. Thompson, S.L.; Bakhoum, S.F.; Compton, D.A. Mechanisms of chromosomal instability. *Curr Biol* **2010**, *20*, R285-295.
20. Cimini, D.; Tanzarella, C.; Degrassi, F. Differences in malsegregation rates obtained by scoring ana-telophases or binucleate cells. *Mutagenesis* **1999**, *14*, 563-568.
21. Thompson, S.L.; Compton, D.A. Examining the link between chromosomal instability and aneuploidy in human cells. *J Cell Biol* **2008**, *180*, 665-672.
22. Lara-Gonzalez, P.; Westhorpe, F.G.; Taylor, S.S. The spindle assembly checkpoint. *Curr Biol* **2012**, *22*, R966-980.
23. Dobles, M.; Liberal, V.; Scott, M.L.; Ben Ezra, R.; Sorger, P.K. Chromosome missegregation and apoptosis in mice lacking the mitotic checkpoint protein MAD2. *Cell* **2000**, *101*, 635-645.



24. Rieder, C.L.; Schultz, A.; Cole, R.; Sluder, G. Anaphase onset in vertebrate somatic cells is controlled by a checkpoint that monitors sister kinetochore attachment to the spindle. *J Cell Biol* **1994**, *127*, 1301-1310.
25. Rieder, C.L.; Cole, R.W.; Khodjakov, A.; Sluder, G. The checkpoint delaying anaphase in response to chromosome monoorientation is mediated by an inhibitory signal produced by unattached kinetochores. *J Cell Biol* **1995**, *130*, 941-948.
26. Khodjakov, A.; Cole, R.W.; McEwen, B.F.; Buttle, K.F.; Rieder, C.L. Chromosome fragments possessing only one kinetochore can congress to the spindle equator. *J Cell Biol* **1997**, *136*, 229-240.
27. Cimini, D.; Fioravanti, D.; Salmon, E.D.; Degrassi, F. Merotelic kinetochore orientation versus chromosome mono-orientation in the origin of lagging chromosomes in human primary cells. *J Cell Sci* **2002**, *115*, 507-515.
28. Gregan, J.; Polakova, S.; Zhang, L.; Tolic-Norrelykke, I.M.; Cimini, D. Merotelic kinetochore attachment: Causes and effects. *Trends Cell Biol* **2011**, *21*, 374-381.
29. Martinez, A.C.; van Wely, K.H. Are aneuploidy and chromosome breakage caused by a single mechanism? *Cell Cycle* **2010**, *9*, 2275-2280.
30. Hanahan, D.; Weinberg, R.A. Hallmarks of cancer: The next generation. *Cell* **2011**, *144*, 646-674.
31. Michor, F.; Iwasa, Y.; Vogelstein, B.; Lengauer, C.; Nowak, M.A. Can chromosomal instability initiate tumorigenesis? *Semin Cancer Biol* **2005**, *15*, 43-49.
32. Chen, J.; Fu, L.; Zhang, L.Y.; Kwong, D.L.; Yan, L.; Guan, X.Y. Tumor suppressor genes on frequently deleted chromosome 3p in nasopharyngeal carcinoma. *Chin J Cancer* **2012**, *31*, 215-222.
33. Kops, G.J.; Weaver, B.A.; Cleveland, D.W. On the road to cancer: Aneuploidy and the mitotic checkpoint. *Nat Rev Cancer* **2005**, *5*, 773-785.
34. Giam, M.; Rancati, G. Aneuploidy and chromosomal instability in cancer: A jackpot to chaos. *Cell Div* **2015**, *10*, 3.
35. Potapova, T.A.; Zhu, J.; Li, R. Aneuploidy and chromosomal instability: A vicious cycle driving cellular evolution and cancer genome chaos. *Cancer Metastasis Rev* **2013**, *32*, 377-389.
36. Heng, H.H.; Bremer, S.W.; Stevens, J.B.; Horne, S.D.; Liu, G.; Abdallah, B.Y.; Ye, K.J.; Ye, C.J. Chromosomal instability (cin): What it is and why it is crucial to cancer evolution. *Cancer Metastasis Rev* **2013**, *32*, 325-340.
37. Chandrakasan, S.; Ye, C.J.; Chitlur, M.; Mohamed, A.N.; Rabah, R.; Konski, A.; Heng, H.H.; Savasan, S. Malignant fibrous histiocytoma two years after autologous stem cell transplant for hodgkin lymphoma: Evidence for genomic instability. *Pediatr Blood Cancer* **2011**, *56*, 1143-1145.
38. Thompson, S.L.; Compton, D.A. Chromosomes and cancer cells. *Chromosome Res* **2011**, *19*, 433-444.
39. Walther, A.; Houlston, R.; Tomlinson, I. Association between chromosomal instability and prognosis in colorectal cancer: A meta-analysis. *Gut* **2008**, *57*, 941-950.
40. Florl, A.R.; Schulz, W.A. Chromosomal instability in bladder cancer. *Arch Toxicol* **2008**, *82*, 173-182.
41. Diaz, L.A., Jr. The current clinical value of genomic instability. *Semin Cancer Biol* **2005**, *15*, 67-71.
42. Orsetti, B.; Selves, J.; Bascoul-Mollevi, C.; Lasorsa, L.; Gordien, K.; Bibeau, F.; Massemin, B.; Paraf, F.; Soubeyran, I.; Hostein, I., et al. Impact of



- chromosomal instability on colorectal cancer progression and outcome. *BMC Cancer* **2014**, *14*, 121.
43. Ferlay, J.; Soerjomataram, I.; Dikshit, R.; Eser, S.; Mathers, C.; Rebelo, M.; Parkin, D.M.; Forman, D.; Bray, F. Cancer incidence and mortality worldwide: Sources, methods and major patterns in globocan 2012. *Int J Cancer* **2015**, *136*, E359-386.
 44. Kwei, K.A.; Kung, Y.; Salari, K.; Holcomb, I.N.; Pollack, J.R. Genomic instability in breast cancer: Pathogenesis and clinical implications. *Mol Oncol* **2010**, *4*, 255-266.
 45. Tsarouha, H.; Pandis, N.; Bardi, G.; Teixeira, M.R.; Andersen, J.A.; Heim, S. Karyotypic evolution in breast carcinomas with i(1)(q10) and der(1;16)(q10;p10) as the primary chromosome abnormality. *Cancer Genet Cytogenet* **1999**, *113*, 156-161.
 46. Cummings, M.C.; Aubele, M.; Mattis, A.; Purdie, D.; Hutzler, P.; Hofler, H.; Werner, M. Increasing chromosome 1 copy number parallels histological progression in breast carcinogenesis. *Br J Cancer* **2000**, *82*, 1204-1210.
 47. Rye, I.H.; Lundin, P.; Maner, S.; Fjelldal, R.; Naume, B.; Wigler, M.; Hicks, J.; Borresen-Dale, A.L.; Zetterberg, A.; Russnes, H.G. Quantitative multigene fish on breast carcinomas identifies der(1;16)(q10;p10) as an early event in luminal a tumors. *Genes Chromosomes Cancer* **2015**, *54*, 235-248.
 48. Perou, C.M.; Sorlie, T.; Eisen, M.B.; van de Rijn, M.; Jeffrey, S.S.; Rees, C.A.; Pollack, J.R.; Ross, D.T.; Johnsen, H.; Akslen, L.A., et al. Molecular portraits of human breast tumours. *Nature* **2000**, *406*, 747-752.
 49. Sorlie, T.; Perou, C.M.; Tibshirani, R.; Aas, T.; Geisler, S.; Johnsen, H.; Hastie, T.; Eisen, M.B.; van de Rijn, M.; Jeffrey, S.S., et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci USA* **2001**, *98*, 10869-10874.
 50. Chin, K.; DeVries, S.; Fridlyand, J.; Spellman, P.T.; Roydasgupta, R.; Kuo, W.L.; Lapuk, A.; Neve, R.M.; Qian, Z.; Ryder, T., et al. Genomic and transcriptional aberrations linked to breast cancer pathophysiologies. *Cancer Cell* **2006**, *10*, 529-541.
 51. Simpson, P.T.; Gale, T.; Reis-Filho, J.S.; Jones, C.; Parry, S.; Sloane, J.P.; Hanby, A.; Pinder, S.E.; Lee, A.H.; Humphreys, S., et al. Columnar cell lesions of the breast: The missing link in breast cancer progression? A morphological and molecular analysis. *Am J Surg Pathol* **2005**, *29*, 734-746.
 52. Courjal, F.; Cuny, M.; Simony-Lafontaine, J.; Louason, G.; Speiser, P.; Zeillinger, R.; Rodriguez, C.; Theillet, C. Mapping of DNA amplifications at 15 chromosomal localizations in 1875 breast tumors: Definition of phenotypic groups. *Cancer Res* **1997**, *57*, 4360-4367.
 53. Smid, M.; Hoes, M.; Siewerts, A.M.; Sleijfer, S.; Zhang, Y.; Wang, Y.; Foekens, J.A.; Martens, J.W. Patterns and incidence of chromosomal instability and their prognostic relevance in breast cancer subtypes. *Breast Cancer Res Treat* **2011**, *128*, 23-30.
 54. Hudis, C.A. Trastuzumab--mechanism of action and use in clinical practice. *N Engl J Med* **2007**, *357*, 39-51.
 55. Gibbs, M.; Stanford, J.L.; McIndoe, R.A.; Jarvik, G.P.; Kolb, S.; Goode, E.L.; Chakrabarti, L.; Schuster, E.F.; Buckley, V.A.; Miller, E.L., et al. Evidence for a rare prostate cancer-susceptibility locus at chromosome 1p36. *Am J Hum Genet* **1999**, *64*, 776-787.
 56. Ribeiro, F.R.; Jeronimo, C.; Henrique, R.; Fonseca, D.; Oliveira, J.; Lothe, R.A.; Teixeira, M.R. 8q gain is an independent predictor of poor survival in diagnostic



- needle biopsies from prostate cancer suspects. *Clin Cancer Res* **2006**, *12*, 3961-3970.
57. Wolter, H.; Trijic, D.; Gottfried, H.W.; Mattfeldt, T. Chromosomal changes in incidental prostatic carcinomas detected by comparative genomic hybridization. *Eur Urol* **2002**, *41*, 328-334.
58. Al-Maghribi, J.; Vorobyova, L.; Chapman, W.; Jewett, M.; Zielenska, M.; Squire, J.A. P53 alteration and chromosomal instability in prostatic high-grade intraepithelial neoplasia and concurrent carcinoma: Analysis by immunohistochemistry, interphase *in situ* hybridization, and sequencing of laser-captured microdissected specimens. *Mod Pathol* **2001**, *14*, 1252-1262.
59. El-Zein, R.; Gu, Y.; Sierra, M.S.; Spitz, M.R.; Strom, S.S. Chromosomal instability in peripheral blood lymphocytes and risk of prostate cancer. *Cancer Epidemiol Biomarkers Prev* **2005**, *14*, 748-752.
60. Bonassi, S.; Hagmar, L.; Stromberg, U.; Montagud, A.H.; Tinnerberg, H.; Forni, A.; Heikkila, P.; Wanders, S.; Wilhardt, P.; Hansteen, I.L., et al. Chromosomal aberrations in lymphocytes predict human cancer independently of exposure to carcinogens. European study group on cytogenetic biomarkers and health. *Cancer Res* **2000**, *60*, 1619-1625.
61. Holcomb, I.N.; Grove, D.I.; Kinnunen, M.; Friedman, C.L.; Gallaher, I.S.; Morgan, T.M.; Sather, C.L.; Delrow, J.J.; Nelson, P.S.; Lange, P.H., et al. Genomic alterations indicate tumor origin and varied metastatic potential of disseminated cells from prostate cancer patients. *Cancer Res* **2008**, *68*, 5599-5608.
62. Visakorpi, T.; Kallioniemi, A.H.; Syvanen, A.C.; Hyttinen, E.R.; Karhu, R.; Tammela, T.; Isola, J.J.; Kallioniemi, O.P. Genetic changes in primary and recurrent prostate cancer by comparative genomic hybridization. *Cancer Res* **1995**, *55*, 342-347.
63. Baca, S.C.; Prandi, D.; Lawrence, M.S.; Mosquera, J.M.; Romanel, A.; Drier, Y.; Park, K.; Kitabayashi, N.; MacDonald, T.Y.; Ghandi, M., et al. Punctuated evolution of prostate cancer genomes. *Cell* **2013**, *153*, 666-677.
64. Fearon, E.R.; Vogelstein, B. A genetic model for colorectal tumorigenesis. *Cell* **1990**, *61*, 759-767.
65. Keino-Masu, K.; Masu, M.; Hinck, L.; Leonardo, E.D.; Chan, S.S.; Culotti, J.G.; Tessier-Lavigne, M. Deleted in colorectal cancer (dcc) encodes a netrin receptor. *Cell* **1996**, *87*, 175-185.
66. Fazeli, A.; Dickinson, S.L.; Hermiston, M.L.; Tighe, R.V.; Steen, R.G.; Small, C.G.; Stoeckli, E.T.; Keino-Masu, K.; Masu, M.; Rayburn, H., et al. Phenotype of mice lacking functional deleted in colorectal cancer (dcc) gene. *Nature* **1997**, *386*, 796-804.
67. Takagi, Y.; Kohmura, H.; Futamura, M.; Kida, H.; Tanemura, H.; Shimokawa, K.; Saji, S. Somatic alterations of the dpc4 gene in human colorectal cancers *in vivo*. *Gastroenterology* **1996**, *111*, 1369-1372.
68. Takagi, Y.; Koumura, H.; Futamura, M.; Aoki, S.; Ymaguchi, K.; Kida, H.; Tanemura, H.; Shimokawa, K.; Saji, S. Somatic alterations of the SMAD-2 gene in human colorectal cancers. *Br J Cancer* **1998**, *78*, 1152-1155.
69. Shih, I.M.; Zhou, W.; Goodman, S.N.; Lengauer, C.; Kinzler, K.W.; Vogelstein, B. Evidence that genetic instability occurs at an early stage of colorectal tumorigenesis. *Cancer Res* **2001**, *61*, 818-822.
70. Cardoso, J.; Molenaar, L.; de Menezes, R.X.; van Leerdam, M.; Rosenberg, C.; Moslein, G.; Sampson, J.; Morreau, H.; Boer, J.M.; Fodde, R. Chromosomal instability in MYH- and APC-mutant adenomatous polyps. *Cancer Res* **2006**, *66*, 2514-2519.



71. Gutenberg, A.; Gerdes, J.S.; Jung, K.; Sander, B.; Gunawan, B.; Bock, H.C.; Liersch, T.; Bruck, W.; Rohde, V.; Fuzesi, L. High chromosomal instability in brain metastases of colorectal carcinoma. *Cancer Genet Cytogenet* **2010**, *198*, 47-51.
72. Swanton, C.; Marani, M.; Pardo, O.; Warne, P.H.; Kelly, G.; Sahai, E.; Elustondo, F.; Chang, J.; Temple, J.; Ahmed, A.A., et al. Regulators of mitotic arrest and ceramide metabolism are determinants of sensitivity to paclitaxel and other chemotherapeutic drugs. *Cancer Cell* **2007**, *11*, 498-512.
73. Giovinazzi, S.; Lindsay, C.R.; Morozov, V.M.; Escobar-Cabrera, E.; Summers, M.K.; Han, H.S.; McIntosh, L.P.; Ishov, A.M. Regulation of mitosis and taxane response by DAXX and RASSF1. *Oncogene* **2012**, *31*, 13-26.
74. How, C.; Bruce, J.; So, J.; Pintilie, M.; Haibe-Kains, B.; Hui, A.; Clarke, B.A.; Hedley, D.W.; Hill, R.P.; Milosevic, M., et al. Chromosomal instability as a prognostic marker in cervical cancer. *BMC Cancer* **2015**, *15*, 361.
75. Singh, M.; Mehrotra, S.; Kalra, N.; Singh, U.; Shukla, Y. Correlation of DNA ploidy with progression of cervical cancer. *J Cancer Epidemiol* **2008**, *2008*, 298495.
76. Olaharski, A.J.; Sotelo, R.; Solorza-Luna, G.; Gonsebatt, M.E.; Guzman, P.; Mohar, A.; Eastmond, D.A. Tetraploidy and chromosomal instability are early events during cervical carcinogenesis. *Carcinogenesis* **2006**, *27*, 337-343.
77. Cortes-Gutierrez, E.I.; Davila-Rodriguez, M.I.; Muraira-Rodriguez, M.; Said-Fernandez, S.; Cerda-Flores, R.M. Association between the stages of cervical cancer and chromosome 1 aneusomy. *Cancer Genet Cytogenet* **2005**, *159*, 44-47.
78. Wang, X.; Zheng, B.; Zhang, R.R.; Li, S.; Chen, X.; Mulvihill, J.J.; Lu, X.; Pang, H.; Liu, H. Automated analysis of fluorescent in situ hybridization (fish) labeled genetic biomarkers in assisting cervical cancer diagnosis. *Technol Cancer Res Treat* **2010**, *9*, 231-242.
79. Murty, V.V.; Mitra, A.B.; Luthra, U.K. Spontaneous chromosomal aberrations in patients with precancerous and cancerous lesions of the cervix uteri. *Cancer Genet Cytogenet* **1985**, *17*, 347-353.
80. Susini, T.; Olivieri, S.; Molino, C.; Amunni, G.; Rapi, S.; Taddei, G.; Scarselli, G. DNA ploidy is stronger than lymph node metastasis as prognostic factor in cervical carcinoma: 10-year results of a prospective study. *Int J Gynecol Cancer* **2011**, *21*, 678-684.
81. Luzhna, L.; Kathiria, P.; Kovalchuk, O. Micronuclei in genotoxicity assessment: From genetics to epigenetics and beyond. *Front Genet* **2013**, *4*, 131.
82. Nersesyan, A.K. Possible role of the micronucleus assay in diagnostics and secondary prevention of cervix cancer: A minireview. *Tsitol Genet* **2007**, *41*, 64-66.
83. Guzman, P.; Sotelo-Regil, R.C.; Mohar, A.; Gonsebatt, M.E. Positive correlation between the frequency of micronucleated cells and dysplasia in papanicolaou smears. *Environ Mol Mutagen* **2003**, *41*, 339-343.
84. Cortes-Gutierrez, E.I.; D'Avila-Rodriguez, M.I.; Cerda-Flores, R.M. Chromosomal damage as prognosis marker in cervical carcinogenesis. *Tsitol Genet* **2014**, *48*, 54-63.
85. Weiderpass, E.; Labreche, F. Malignant tumors of the female reproductive system. *Saf Health Work* **2012**, *3*, 166-180.
86. Sonoda, G.; du Manoir, S.; Godwin, A.K.; Bell, D.W.; Liu, Z.; Hogan, M.; Yakushiji, M.; Testa, J.R. Detection of DNA gains and losses in primary endometrial carcinomas by comparative genomic hybridization. *Genes Chromosomes Cancer* **1997**, *18*, 115-125.



87. Kinzler, K.W.; Vogelstein, B. Cancer-susceptibility genes. Gatekeepers and caretakers. *Nature* **1997**, *386*, 761, 763.
88. Peiffer-Schneider, S.; Noonan, F.C.; Mutch, D.G.; Simpkins, S.B.; Herzog, T.; Rader, J.; Elbendary, A.; Gersell, D.J.; Call, K.; Goodfellow, P.J. Mapping an endometrial cancer tumor suppressor gene at 10q25 and development of a bacterial clone contig for the consensus deletion interval. *Genomics* **1998**, *52*, 9-16.
89. Sirchia, S.M.; Pariani, S.; Rossella, F.; Garagiola, I.; De Andreis, C.; Bulfamante, G.; Zannoni, E.; Radaelli, U.; Simoni, G. Cytogenetic abnormalities and microsatellite instability in endometrial adenocarcinoma. *Cancer Genet Cytogenet* **1997**, *94*, 113-119.
90. Muresu, R.; Sini, M.C.; Cossu, A.; Tore, S.; Baldinu, P.; Manca, A.; Pisano, M.; Loddo, C.; Dessole, S.; Pintus, A., et al. Chromosomal abnormalities and microsatellite instability in sporadic endometrial cancer. *Eur J Cancer* **2002**, *38*, 1802-1809.
91. Peterson, L.M.; Kipp, B.R.; Halling, K.C.; Kerr, S.E.; Smith, D.I.; Distad, T.J.; Clayton, A.C.; Medeiros, F. Molecular characterization of endometrial cancer: A correlative study assessing microsatellite instability, mlh1 hypermethylation, DNA mismatch repair protein expression, and pten, pik3ca, kras, and braf mutation analysis. *Int J Gynecol Pathol* **2012**, *31*, 195-205.
92. Bochkov, N.P.; Chebotarev, A.N.; Katosova, L.D.; Platonova, V.I. [the database for analysis of quantitative characteristics of chromosome aberration frequencies in the culture of human peripheral blood lymphocytes]. *Genetika* **2001**, *37*, 549-557.
93. Nesina, I.P.; Iurchenko, N.P.; Nespryadko, S.V.; Buchinska, L.G. The study of chromosomal instability in patients with endometrial cancer. *Exp Oncol* **2014**, *36*, 202-206.
94. Pilinska, M.A.; Dybsky, S.S.; Dybska, O.B.; Shvayko, L.I.; Sushko, V.O. Peculiarities of induction and persistence of hidden chromosome instability in peripheral blood lymphocytes of persons occupationally exposed to ionizing radiation. *Probl Radiac Med Radiobiol* **2014**, *19*, 321-333.
95. Abraham, R.; Pagano, F.; Gomella, L.G.; Baffa, R. Chromosomal deletions in bladder cancer: Shutting down pathways. *Front Biosci* **2007**, *12*, 826-838.
96. Cairns, P.; Shaw, M.E.; Knowles, M.A. Preliminary mapping of the deleted region of chromosome 9 in bladder cancer. *Cancer Res* **1993**, *53*, 1230-1232.
97. Sauter, G.; Moch, H.; Wagner, U.; Bubendorf, L.; Gasser, T.C.; Mihatsch, M.J. [genomic changes in urinary bladder cancer]. *Verh Dtsch Ges Pathol* **1997**, *81*, 287-296.
98. Sauter, G.; Simon, R.; Bubendorf, L.; Mihatsch, M. [molecular genetics of urinary bladder cancer progression]. *Verh Dtsch Ges Pathol* **2002**, *86*, 49-56.
99. Kimura, F.; Florl, A.R.; Seifert, H.H.; Louhelainen, J.; Maas, S.; Knowles, M.A.; Schulz, W.A. Destabilization of chromosome 9 in transitional cell carcinoma of the urinary bladder. *Br J Cancer* **2001**, *85*, 1887-1893.
100. Bernues, M.; Casadevall, C.; Caballin, M.R.; Miro, R.; Ejarque, M.J.; Chechile, G.; Gelabert, A.; Egozcue, J. Study of allelic losses on 3p, 6q, and 17p in human urothelial cancer. *Cancer Genet Cytogenet* **1999**, *112*, 42-45.
101. Li, M.; Zhang, Z.F.; Reuter, V.E.; Cordon-Cardo, C. Chromosome 3 allelic losses and microsatellite alterations in transitional cell carcinoma of the urinary bladder. *Am J Pathol* **1996**, *149*, 229-235.
102. Wagner, U.; Bubendorf, L.; Gasser, T.C.; Moch, H.; Gorog, J.P.; Richter, J.; Mihatsch, M.J.; Waldman, F.M.; Sauter, G. Chromosome 8p deletions are



- associated with invasive tumor growth in urinary bladder cancer. *Am J Pathol* **1997**, *151*, 753-759.
103. Choi, C.; Kim, M.H.; Juhng, S.W.; Oh, B.R. Loss of heterozygosity at chromosome segments 8p22 and 8p11.2-21.1 in transitional-cell carcinoma of the urinary bladder. *Int J Cancer* **2000**, *86*, 501-505.
 104. Seripa, D.; Parrella, P.; Gallucci, M.; Gravina, C.; Papa, S.; Fortunato, P.; Alcini, A.; Flammia, G.; Lazzari, M.; Fazio, V.M. Sensitive detection of transitional cell carcinoma of the bladder by microsatellite analysis of cells exfoliated in urine. *Int J Cancer* **2001**, *95*, 364-369.
 105. Panani, A.D.; Babanaraki, A.; Malianga, E.; Roussos, C. Numerical aberrations of chromosomes 9 and 11 detected by fish in greek bladder cancer patients. *Anticancer Res* **2004**, *24*, 3857-3861.
 106. Lokeshwar, V.B.; Habuchi, T.; Grossman, H.B.; Murphy, W.M.; Hautmann, S.H.; Hemstreet, G.P., 3rd; Bono, A.V.; Getzenberg, R.H.; Goebell, P.; Schmitz-Drager, B.J., et al. Bladder tumor markers beyond cytology: International consensus panel on bladder tumor markers. *Urology* **2005**, *66*, 35-63.
 107. Bonberg, N.; Taeger, D.; Gawrych, K.; Johnen, G.; Banek, S.; Schwentner, C.; Sievert, K.D.; Wellhausser, H.; Kluckert, M.; Leng, G., et al. Chromosomal instability and bladder cancer: The urovysion(tm) test in the uroscreen study. *BJU Int* **2013**, *112*, E372-382.
 108. Fonseca, R.; Bergsagel, P.L.; Drach, J.; Shaughnessy, J.; Gutierrez, N.; Stewart, A.K.; Morgan, G.; Van Ness, B.; Chesi, M.; Minvielle, S., et al. International myeloma working group molecular classification of multiple myeloma: Spotlight review. *Leukemia* **2009**, *23*, 2210-2221.
 109. Keats, J.J.; Reiman, T.; Belch, A.R.; Pilarski, L.M. Ten years and counting: So what do we know about t(4;14)(p16;q32) multiple myeloma. *Leuk Lymphoma* **2006**, *47*, 2289-2300.
 110. Boyd, K.D.; Ross, F.M.; Tapper, W.J.; Chieccchio, L.; Dagrada, G.; Konn, Z.J.; Gonzalez, D.; Walker, B.A.; Hockley, S.L.; Wardell, C.P., et al. The clinical impact and molecular biology of del(17p) in multiple myeloma treated with conventional or thalidomide-based therapy. *Genes Chromosomes Cancer* **2011**, *50*, 765-774.
 111. Fonseca, R.; Blood, E.; Rue, M.; Harrington, D.; Oken, M.M.; Kyle, R.A.; Dewald, G.W.; Van Ness, B.; Van Wier, S.A.; Henderson, K.J., et al. Clinical and biologic implications of recurrent genomic aberrations in myeloma. *Blood* **2003**, *101*, 4569-4575.
 112. Harousseau, J.L.; Avet-Loiseau, H.; Attal, M.; Charbonnel, C.; Garban, F.; Hulin, C.; Michallet, M.; Facon, T.; Garderet, L.; Marit, G., et al. Achievement of at least very good partial response is a simple and robust prognostic factor in patients with multiple myeloma treated with high-dose therapy: Long-term analysis of the ifm 99-02 and 99-04 trials. *J Clin Oncol* **2009**, *27*, 5720-5726.
 113. Chung, T.H.; Mulligan, G.; Fonseca, R.; Chng, W.J. A novel measure of chromosome instability can account for prognostic difference in multiple myeloma. *PLoS one* **2013**, *8*, e66361.
 114. Paulsson, K.; Johansson, B. High hyperdiploid childhood acute lymphoblastic leukemia. *Genes Chromosomes Cancer* **2009**, *48*, 637-660.
 115. Heerema, N.A.; Raimondi, S.C.; Anderson, J.R.; Biegel, J.; Camitta, B.M.; Cooley, L.D.; Gaynon, P.S.; Hirsch, B.; Magenis, R.E.; McGavran, L., et al. Specific extra chromosomes occur in a modal number dependent pattern in pediatric acute lymphoblastic leukemia. *Genes Chromosomes Cancer* **2007**, *46*, 684-693.



116. Moorman, A.V.; Clark, R.; Farrell, D.M.; Hawkins, J.M.; Martineau, M.; Secker-Walker, L.M. Probes for hidden hyperdiploidy in acute lymphoblastic leukaemia. *Genes Chromosomes Cancer* **1996**, *16*, 40-45.
117. Paulsson, K.; Morse, H.; Fioretos, T.; Behrendtz, M.; Strombeck, B.; Johansson, B. Evidence for a single-step mechanism in the origin of hyperdiploid childhood acute lymphoblastic leukemia. *Genes Chromosomes Cancer* **2005**, *44*, 113-122.
118. Forestier, E.; Johansson, B.; Borgstrom, G.; Kerndrup, G.; Johansson, J.; Heim, S. Cytogenetic findings in a population-based series of 787 childhood acute lymphoblastic leukemias from the nordic countries. The nopho leukemia cytogenetic study group. *Eur J Haematol* **2000**, *64*, 194-200.
119. Moorman, A.V.; Richards, S.M.; Martineau, M.; Cheung, K.L.; Robinson, H.M.; Jalali, G.R.; Broadfield, Z.J.; Harris, R.L.; Taylor, K.E.; Gibson, B.E., et al. Outcome heterogeneity in childhood high-hyperdiploid acute lymphoblastic leukemia. *Blood* **2003**, *102*, 2756-2762.
120. Mitelman F, J.B.a.M.F.E. Mitelman database of chromosome aberrations and gene fusions in cancer (2017). 2017.
121. Blandin, A.T.; Muhlematter, D.; Bougeon, S.; Gogniat, C.; Porter, S.; Beyer, V.; Parlier, V.; Beckmann, J.S.; van Melle, G.; Jotterand, M. Automated four-color interphase fluorescence *in situ* hybridization approach for the simultaneous detection of specific aneuploidies of diagnostic and prognostic significance in high hyperdiploid acute lymphoblastic leukemia. *Cancer Genet Cytogenet* **2008**, *186*, 69-77.
122. Betts, D.R.; Riesch, M.; Grotzer, M.A.; Niggli, F.K. The investigation of karyotypic instability in the high-hyperdiploidy subgroup of acute lymphoblastic leukemia. *Leuk Lymphoma* **2001**, *42*, 187-193.
123. Talamo, A.; Chalandon, Y.; Marazzi, A.; Jotterand, M. Clonal heterogeneity and chromosomal instability at disease presentation in high hyperdiploid acute lymphoblastic leukemia. *Cancer Genet Cytogenet* **2010**, *203*, 209-214.
124. Heerema, N.A.; Sather, H.N.; Sensel, M.G.; Zhang, T.; Hutchinson, R.J.; Nachman, J.B.; Lange, B.J.; Steinherz, P.G.; Bostrom, B.C.; Reaman, G.H., et al. Prognostic impact of trisomies of chromosomes 10, 17, and 5 among children with acute lymphoblastic leukemia and high hyperdiploidy (> 50 chromosomes). *J Clin Oncol* **2000**, *18*, 1876-1887.
125. Dayal, J.; Albergant, L.; Newman, T.; South, A. Quantitation of multiclarity in control and drug-treated tumour populations using high-throughput analysis of karyotypic heterogeneity. *Converg. Sci. Phys. Oncol* **2015**, *1*.
126. Fedorenko, I.V.; Wargo, J.A.; Flaherty, K.T.; Messina, J.L.; Smalley, K.S. Braf inhibition generates a host-tumor niche that mediates therapeutic escape. *J Invest Dermatol* **2015**, *135*, 3115-3124.
127. McGranahan, N.; Burrell, R.A.; Endesfelder, D.; Novelli, M.R.; Swanton, C. Cancer chromosomal instability: Therapeutic and diagnostic challenges. *EMBO Rep* **2012**, *13*, 528-538.
128. Thompson, L.L.; Jeusset, L.M.; Lepage, C.C.; McManus, K.J. Evolving therapeutic strategies to exploit chromosome instability in cancer. *Cancers (Basel)* **2017**, *9*.
129. Horne, S.; Wexler, M.; Stevens, J.; Heng, H.H. Insights on processes of evolutionary tumor growth. *Atlas of Genetics and Cytogenetics in Oncology and Haematology*: 2015.
130. Lee, A.J.; Endesfelder, D.; Rowan, A.J.; Walther, A.; Birkbak, N.J.; Futreal, P.A.; Downward, J.; Szallasi, Z.; Tomlinson, I.P.; Howell, M., et al.



- Chromosomal instability confers intrinsic multidrug resistance. *Cancer Res* **2011**, *71*, 1858-1870.
131. Penner-Goeke, S.; Lichtensztein, Z.; Neufeld, M.; Ali, J.L.; Altman, A.D.; Nachtigal, M.W.; McManus, K.J. The temporal dynamics of chromosome instability in ovarian cancer cell lines and primary patient samples. *PLoS Genet* **2017**, *13*, e1006707.
 132. Cyll, K.; Ersvaer, E.; Vlatkovic, L.; Pradhan, M.; Kildal, W.; Avranden Kjaer, M.; Kleppe, A.; Hveem, T.S.; Carlsen, B.; Gill, S., et al. Tumour heterogeneity poses a significant challenge to cancer biomarker research. *Br J Cancer* **2017**, *117*, 367-375.
 133. Xu, Y.; Qin, T.; Li, J.; Wang, X.; Gao, C.; Xu, C.; Hao, J.; Liu, J.; Gao, S.; Ren, H. Detection of circulating tumor cells using negative enrichment immunofluorescence and an in situ hybridization system in pancreatic cancer. *Int J Mol Sci* **2017**, *18*.
 134. Stomornjak-Vukadin, M.; Kurtovic-Basic, I.; Mehinovic, L.; Konjhodzic, R. Combined use of cytogenetic and molecular methods in prenatal diagnostics of chromosomal abnormalities. *Acta Inform Med* **2015**, *23*, 68-72.
 135. Roylance, R.; Endesfelder, D.; Gorman, P.; Burrell, R.A.; Sander, J.; Tomlinson, I.; Hanby, A.M.; Speirs, V.; Richardson, A.L.; Birkbak, N.J., et al. Relationship of extreme chromosomal instability with long-term survival in a retrospective analysis of primary breast cancer. *Cancer Epidemiol Biomarkers Prev* **2011**, *20*, 2183-2194.
 136. Swanton, C.; Nicke, B.; Schuett, M.; Eklund, A.C.; Ng, C.; Li, Q.; Hardcastle, T.; Lee, A.; Roy, R.; East, P., et al. Chromosomal instability determines taxane response. *Proc Natl Acad Sci USA* **2009**, *106*, 8671-8676.
 137. Jamal-Hanjani, M.; Wilson, G.A.; McGranahan, N.; Birkbak, N.J.; Watkins, T.B.K.; Veeriah, S.; Shafi, S.; Johnson, D.H.; Mitter, R.; Rosenthal, R., et al. Tracking the evolution of non-small-cell lung cancer. *N Engl J Med* **2017**, *376*, 2109-2121.
 138. Kaelin, W.G., Jr. The concept of synthetic lethality in the context of anticancer therapy. *Nat Rev Cancer* **2005**, *5*, 689-698.
 139. Farmer, H.; McCabe, N.; Lord, C.J.; Tutt, A.N.; Johnson, D.A.; Richardson, T.B.; Santarosa, M.; Dillon, K.J.; Hickson, I.; Knights, C., et al. Targeting the DNA repair defect in brca mutant cells as a therapeutic strategy. *Nature* **2005**, *434*, 917-921.
 140. Bryant, H.E.; Schultz, N.; Thomas, H.D.; Parker, K.M.; Flower, D.; Lopez, E.; Kyle, S.; Meuth, M.; Curtin, N.J.; Helleday, T. Specific killing of BRCA2-deficient tumours with inhibitors of poly(adp-ribose) polymerase. *Nature* **2005**, *434*, 913-917.
 141. Zasadil, L.M.; Andersen, K.A.; Yeum, D.; Rocque, G.B.; Wilke, L.G.; Tevaarwerk, A.J.; Raines, R.T.; Burkard, M.E.; Weaver, B.A. Cytotoxicity of paclitaxel in breast cancer is due to chromosome missegregation on multipolar spindles. *Sci Transl Med* **2014**, *6*, 229ra243.
 142. Kops, G.J.; Foltz, D.R.; Cleveland, D.W. Lethality to human cancer cells through massive chromosome loss by inhibition of the mitotic checkpoint. *Proc Natl Acad Sci USA* **2004**, *101*, 8699-8704.
 143. Janssen, A.; Kops, G.J.; Medema, R.H. Elevating the frequency of chromosome mis-segregation as a strategy to kill tumor cells. *Proc Natl Acad Sci USA* **2009**, *106*, 19108-19113.
 144. Silk, A.D.; Zasadil, L.M.; Holland, A.J.; Vitre, B.; Cleveland, D.W.; Weaver, B.A. Chromosome missegregation rate predicts whether aneuploidy will promote or suppress tumors. *Proc Natl Acad Sci USA* **2013**, *110*, E4134-4141.



145. Chesnokova, V.; Kovacs, K.; Castro, A.V.; Zonis, S.; Melmed, S. Pituitary hypoplasia in pttg-/- mice is protective for rb+/- pituitary tumorigenesis. *Mol Endocrinol* **2005**, *19*, 2371-2379.
146. Pavelka, N.; Rancati, G.; Li, R. Dr jekyll and MR hyde: Role of aneuploidy in cellular adaptation and cancer. *Curr Opin Cell Biol* **2010**, *22*, 809-815.
147. Weaver, B.A.; Cleveland, D.W. Aneuploidy: Instigator and inhibitor of tumorigenesis. *Cancer Res* **2007**, *67*, 10103-10105.
148. Khan, I.; Malinge, S.; Crispino, J. Myeloid leukemia in down syndrome. *Crit Rev Oncog* **2011**, *16*, 25-36.
149. Schoemaker, M.J.; Swerdlow, A.J.; Higgins, C.D.; Wright, A.F.; Jacobs, P.A.; Group, U.K.C.C. Cancer incidence in women with turner syndrome in great britain: A national cohort study. *Lancet Oncol* **2008**, *9*, 239-246.
150. Swerdlow, A.J.; Schoemaker, M.J.; Higgins, C.D.; Wright, A.F.; Jacobs, P.A.; Group, U.K.C.C. Cancer incidence and mortality in men with klinefelter syndrome: A cohort study. *J Natl Cancer Inst* **2005**, *97*, 1204-1210.
151. Higgins, C.D.; Swerdlow, A.J.; Schoemaker, M.J.; Wright, A.F.; Jacobs, P.A.; Group, U.K.C.C. Mortality and cancer incidence in males with y polysomy in britain: A cohort study. *Hum Genet* **2007**, *121*, 691-696.
152. Pinson, L.; Mannini, L.; Willems, M.; Cucco, F.; Sirvent, N.; Frebourg, T.; Quarantotti, V.; Collet, C.; Schneider, A.; Sarda, P., et al. Cep57 mutation in a girl with mosaic variegated aneuploidy syndrome. *Am J Med Genet A* **2014**, *164A*, 177-181.
153. Garcia-Castillo, H.; Vasquez-Velasquez, A.I.; Rivera, H.; Barros-Nunez, P. Clinical and genetic heterogeneity in patients with mosaic variegated aneuploidy: Delineation of clinical subtypes. *Am J Med Genet A* **2008**, *146A*, 1687-1695.
154. Hanks, S.; Coleman, K.; Reid, S.; Plaja, A.; Firth, H.; Fitzpatrick, D.; Kidd, A.; Mehes, K.; Nash, R.; Robin, N., et al. Constitutional aneuploidy and cancer predisposition caused by biallelic mutations in bub1b. *Nat Genet* **2004**, *36*, 1159-1161.
155. Akasaka, N.; Tohyama, J.; Ogawa, A.; Takachi, T.; Watanabe, A.; Asami, K. Refractory infantile spasms associated with mosaic variegated aneuploidy syndrome. *Pediatr Neurol* **2013**, *49*, 364-367.



© 2017 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).



CAPÍTULO III

ROLE OF CHROMOSOMAL INSTABILITY AND CLONAL HETEROGENEITY IN THE THERAPY RESPONSE OF BREAST CANCER CELL LINES



Abstract: Chromosomal instability (CIN) is a hallmark of cancer and a source of genetic variation favoring tumor adaptation to stressful environments. Breast cancer (BC) is characterized by unstable karyotypes and recent reports have indicated that CIN may influence the response of BC tumors to chemotherapy regimens. However, paradoxical associations between extreme CIN and improved outcome have been observed. This study aimed to i) evaluate CIN levels and clonal heterogeneity in MCF7, ZR-751, MDA-MB468, BT474 and KPL4 BC cells treated with low doses of tamoxifen (TAM), docetaxel (DOC), doxorubicin (DOX), Herceptin (HT), and combined treatments (TAM/DOC, TAM/DOX, TAM/HT, HT/DOC and HT/DOX), and ii) examine the association with response to treatments by comparing FISH results with cell proliferation. Intermediate CIN was linked to drug sensitivity according to three characteristics: estrogen receptor β (ER β) and HER2 status, pre-existing CIN level in cancer cells, and the CIN induced by the treatments. ER β +/HER2- cells with intermediate CIN were sensitive to treatment with taxanes (DOC) and anthracyclines (DOX), while ER β -/HER2-, ER β +/HER2+ and ER β -/HER2+ cells with intermediate CIN were resistant to these treatments. A greater understanding of CIN and clonal heterogeneity in BC could assist in the optimization of existing therapeutic regimens and/or in supporting new strategies to improve cancer outcomes.

Keywords: breast cancer; chromosomal instability; therapy resistance; FISH; clonal heterogeneity

1. Introduction

The therapeutic decision regarding estrogen receptor-positive (ER β +) and ER β -negative (ER β -) breast cancer (BC) patients is mainly based on clinical and pathological parameters. In particular, tumor size, histological grade, histotype, immunohistochemical results of prognostic factors and lymph node status play a major role in the selection of therapeutic strategies including hormonal therapy and/or chemotherapy with different agents. Although this has been a successful approach, some patients relapse and/or eventually develop resistance to treatment. Therefore, identifying reliable prognostic and predictive markers is a priority in BC research. A promising therapeutic target is Chromosome Instability (CIN), a common feature in solid tumors [1]. Breast Cancer (BC) is characterized by unstable karyotypes and recent reports have indicated that CIN may influence response to distinct chemotherapy regimens in HER2-positive (HER2+) tumors [1-3].

CIN is characterized by the gain or loss of partial chromosomes or whole chromosomes (aneuploidy) [3]. Aneuploidy refers to the state of abnormal chromosome number, which can be either stable or unstable. Unstable aneuploidy leads to karyotypic heterogeneity between tumor cells [4], which in turn can lead to the simultaneous growth of diverse tumor subpopulations (clonal heterogeneity), resulting in genomic inter- and intra-tumor heterogeneity [3,5]. In addition, both CIN and clonal heterogeneity (CH) have been associated with cancer progression, increased invasiveness and response to therapy [6-8]. Karyotypes of breast tumors are characterized by a high grade of complexity with multiple chromosomes showing both numerical and structural changes. Alterations of chromosome arms 1q, 3p and +7, +8, +20 have been frequently observed in BC, together with cytogenetic signs of DNA amplification, such as homogeneously staining regions that are preferentially associated with 8p and 17q. Moreover, loss of heterozygosity (LOH) and comparative



genomic hybridization (CGH) studies showed deletion and amplification of large genomic segments. Common regions of LOH in BC are located on several chromosomes, including 1p, 1q, 3p, 8p, 11q, 13q, 16q, 17p, 17q, while hot spots for gains are routinely observed at 1q, 8q, 11q, 17q, 20q [9]. Further, cytogenetic and molecular observations show that breast tumours are characterized by multiclarity, suggesting the existence of a high degree of intratumoral heterogeneity, mostly sustained by CIN. CIN and CH lead to gene regulatory interactions and varying protein concentrations, both of which could impact cell responses to drug treatments [10]. Some reports suggest that chromosomal alterations in individual cancer cells induce variable drug sensitivity and thus can lead to the survival of a fraction of tumor cells [11]. For instance, HER2+ tumors with distinct patterns of karyotypic complexity (high CIN) have been associated with sensitivity to anthracycline and platinum-based therapies, while tumors with relative chromosomal stability (low CIN) have been associated with sensitivity to taxanes [12,13]. However, paradoxical relationships between extreme CIN and improved outcome have been observed in patients with various types of cancers, including breast cancer [14,15]. In fact, high CIN has been correlated with improved long-term survival in ER β - BC patients, but poorer outcome in ER β + BC patients [16]. These observations show the complexity associated with selecting appropriate therapies targeting CIN in different types of cancers and suggest that a threshold of CIN may exist that, when exceeded, could induce either cell cytotoxicity or cell survival. However, these CIN thresholds are theoretical and could be specific for each type of cancer.

Taking into account the high frequency of patients who do not respond to therapy or who develop resistance over time, determining and standardizing new prognostic and predictive markers is critical in BC research. Better understanding of the associations between the levels of CIN and CH with the response to therapy may help determine whether there is a critical CIN threshold that distinguishes tumor viability from lethality. These findings could allow for the prediction of the benefit of chemotherapy, hormone therapy and combined therapies in patients with BC of the development of new strategies to improve cancer outcome.

The aim of this study was to determine the CIN level and CH in five human breast cancer cell lines with differential expression of ER β and HER2 and to examine the association with the response to individual treatments, tamoxifen (TAM), docetaxel (DOC), doxorubicin (DOX), and Herceptin (HT), and combined treatments, TAM+DOC, TAM+DOX, TAM+HT, HT+DOC and HT+DOX. We found that intermediate CIN is linked to drug sensitivity according to three characteristics, including ER β and HER2 status, the pre-existing level of CIN of the tumor cells, and the CIN induced by the treatments. In addition, all therapies used in this study (monotherapy or combined therapy) promoted CIN, which influenced the response to therapy.

2. Results

2.1. Definition of CIN levels and CH in control cell lines

Some previous studies in BC patients classified high CIN as CIN >50% [17,18], however we considered CIN >50% as intermediate in this study for all cell lines. The above with the objective of showing more clearly the variations in CIN levels after treatments. Therefore, we considered CIN as high when CIN>70%, as indicated in Materials and Methods.

The CIN for the five breast cancer cell lines analyzed in this study (MCF7, ZR751, MDA-MB468, BT474 and KPL4) ranged from 50% to 64% and the CH ranged from 1,18 to 1,5 (Table S1). These cell lines were defined as having intermediate CIN



and low CH (Figure 1). All untreated cell lines harbored the same level of CIN and CH at 24 h, 48 h and 96 h. High CIN was associated with high CH, which can be observed mainly for chromosomes 2 and 15.

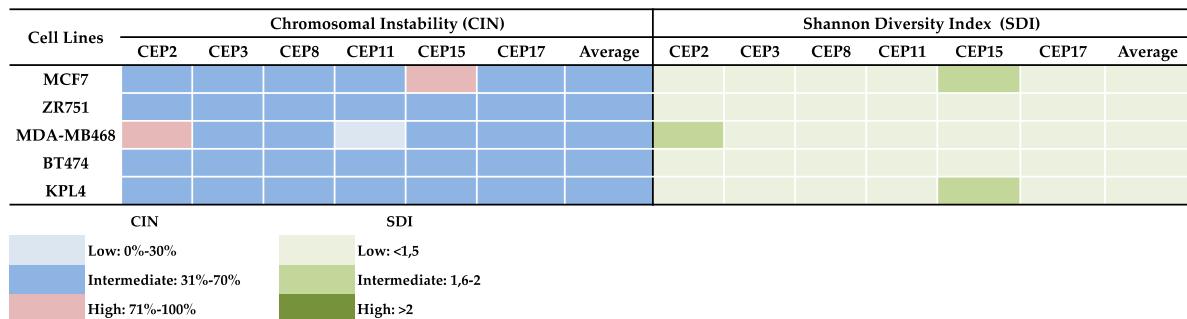


Figure 5. Chromosomal Instability (CIN) and Shannon diversity index (SDI) in untreated breast cancer cell lines. The level of CIN and the SDI (indicative of clonal heterogeneity (CH)) in the three cell lines is color coded according to the legend at the bottom.

2.2. Variation of CIN and CH in HER2- cells after treatments

2.2.1. MCF7 cells

In MCF cells (ER β +/ progesterone receptor (PR)+/HER2-), the best effects in terms of reduction of cell proliferation, were observed when individual and combined treatments, did not increase the CIN above 68% (Figures 2A, 2B and 3, and Tables S1 and S2).

We observed a reduction in cell proliferation in response to all individual and combined treatments (Figures 2A and 2B), and an increase in CIN from 64% in untreated cells to 68% after treatment (Figure 3 and Table S2). Among the monotherapies, DOC induced a greater reduction in cell proliferation (Figure 2A), and also both, an increase in CIN, which did not exceed 68% (Figures 3 and 4), and an increase in CH (from low to intermediate CH). These results show that cells with intermediate CIN respond better to treatment with taxanes (DOC) than with anthracyclines (DOX).

Both combined therapies, TAM+DOC and TAM+DOX, inhibited MCF7 cell proliferation (Table S2), with a greater inhibition when TAM was combined with DOX (TAM+DOX) (Figure 2B). Notably, at 24 h of TAM+DOC, we observed significantly stimulated cell proliferation ($p<0.012$, Student's t-test; Table S2) and increased CIN and CH (CIN= 72% and CH= 1,74) with respect to the untreated cells (CIN=64% and CH=1,5) (Figure 3 and Tables S1 and S2).

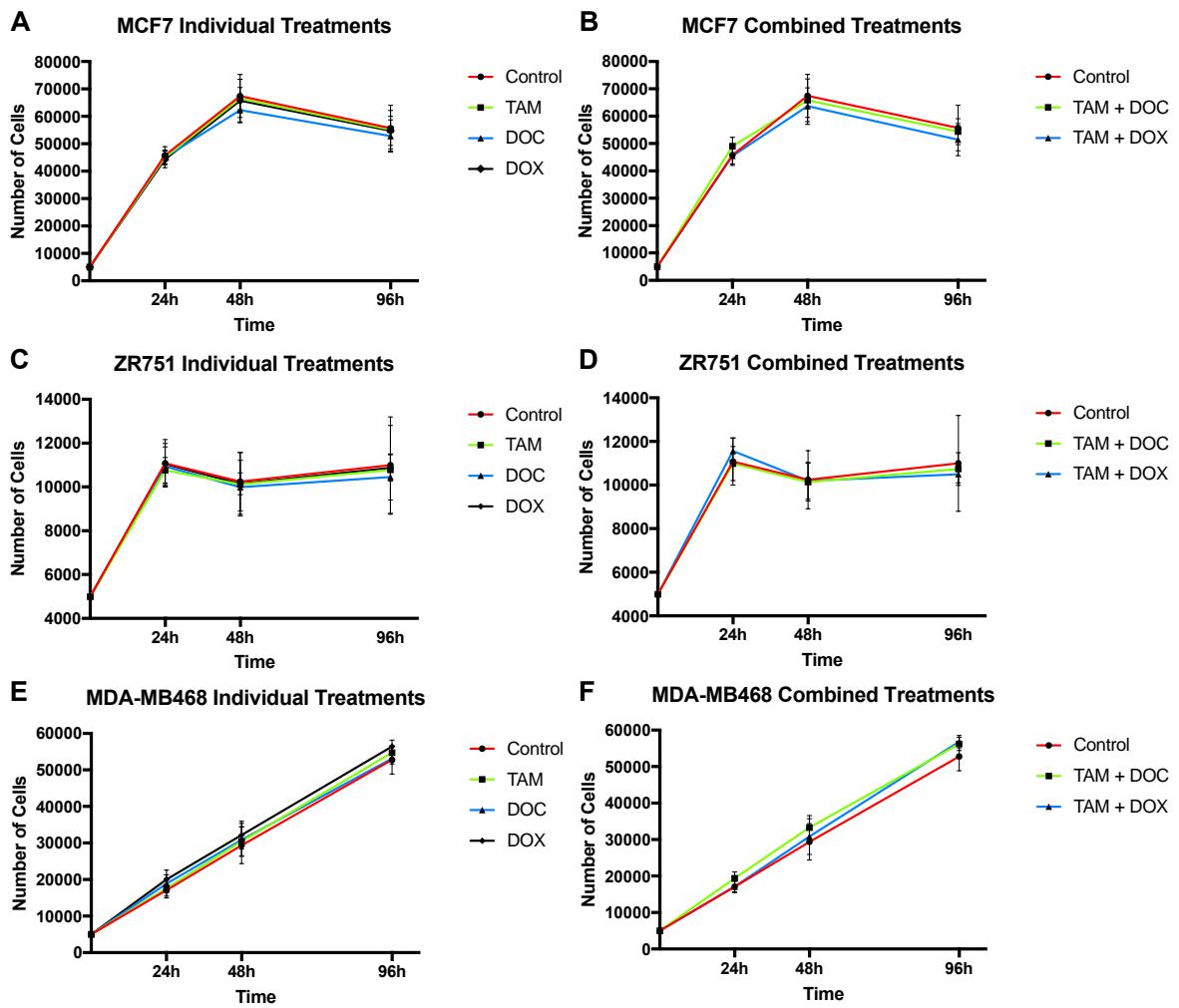


Figure 6. Effects of tamoxifen (TAM), docetaxel (DOC), doxorubicin (DOX), TAM+DOC and TAM+DOX treatments for 24 h, 48 h, and 96 h on cell proliferation in (A–B) MCF7 cells, (C–D) ZR751 cells and (E–F) MDA-MB468 cells. Error bars represent mean standard deviation of 24 separate experiments.

2.2.2. ZR751 cells

In ZR751 cells (ER β +/PR+/HER2-), the best results in terms of reduction of cell proliferation after individual or combined treatments were observed when CIN increased but did not exceed 67% (increased from 50% in untreated cells to 67% after treatments) (Figure 3 and Tables S1 and S3). Among the individual treatments, DOC induced a greater reduction in cell proliferation, and an increase in CIN, which did not exceed 67% (Figure 2C and Figure 3). Similar to results in MCF7 cells, in ZR751 cells, both combined therapies inhibited cell proliferation (Figure 2D and Table S3), and a greater inhibition was observed when TAM was combined with DOX (TAM+DOX) compared with the combination of TAM with DOC. To highlight that in these cells, TAM+DOX treatment for 24 h stimulated cell proliferation (Table S3) and also increased CIN and CH (CIN= 70% and CH=1,7) compared with the control (CIN=50% and CH=1,2) (Figure 3 and Tables S1 and S3).

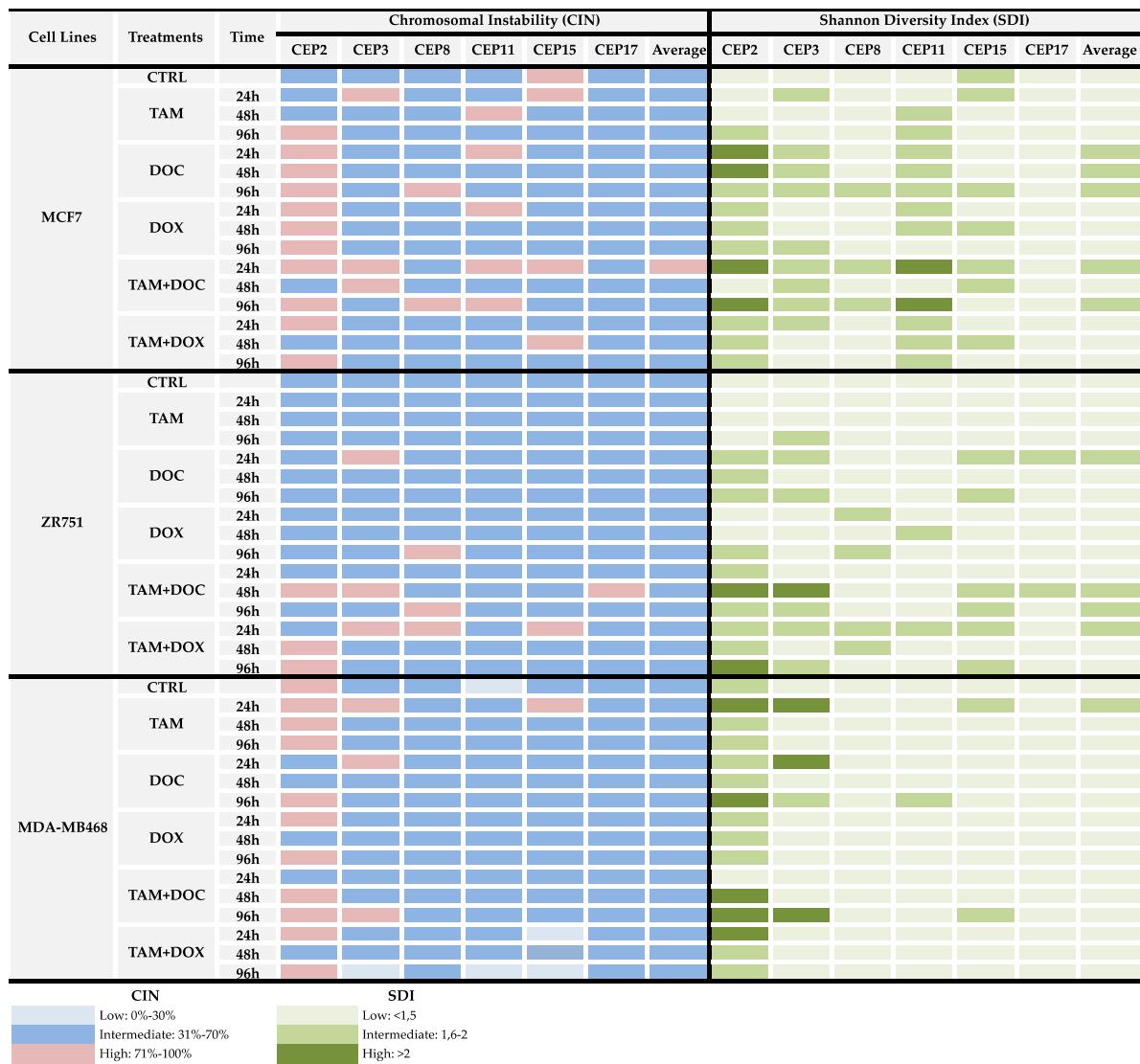


Figure 7. Chromosomal Instability (CIN) and Shannon Diversity Index (SDI) (indicative of clonal heterogeneity) in HER2- breast cancer cells treated with tamoxifen (TAM), docetaxel (DOC), doxorubicin (DOX), TAM+DOC and TAM+DOX at various time points. The level of CIN and the SDI before and after treatments in ER β +PR+/HER2- (MCF7 and ZR751 cells) and ER β -PR-/HER2- (MDA-MB468 cells) cells is color coded according to the legend at the bottom.

These results suggest that ER β +PR+/HER2- tumor cells (MCF7 and ZR751), with intermediate CIN, respond better to treatment with taxanes (DOC) and to combined treatments with TAM+DOX compared with the treatment with anthracyclines and with TAM+DOC. Interestingly, the response is related to an increase in CIN no greater than 68%. In contrast, an increase in CIN equal to or greater than 70% was related to treatment resistance (Tables S1, S2 and S3).

Notably, in all treatments applied and at all times (24 h, 48 h and 96 h), a direct association between CIN and CH was observed, where at higher CIN also a higher CH was observed (Tables S1, S2 and S3).

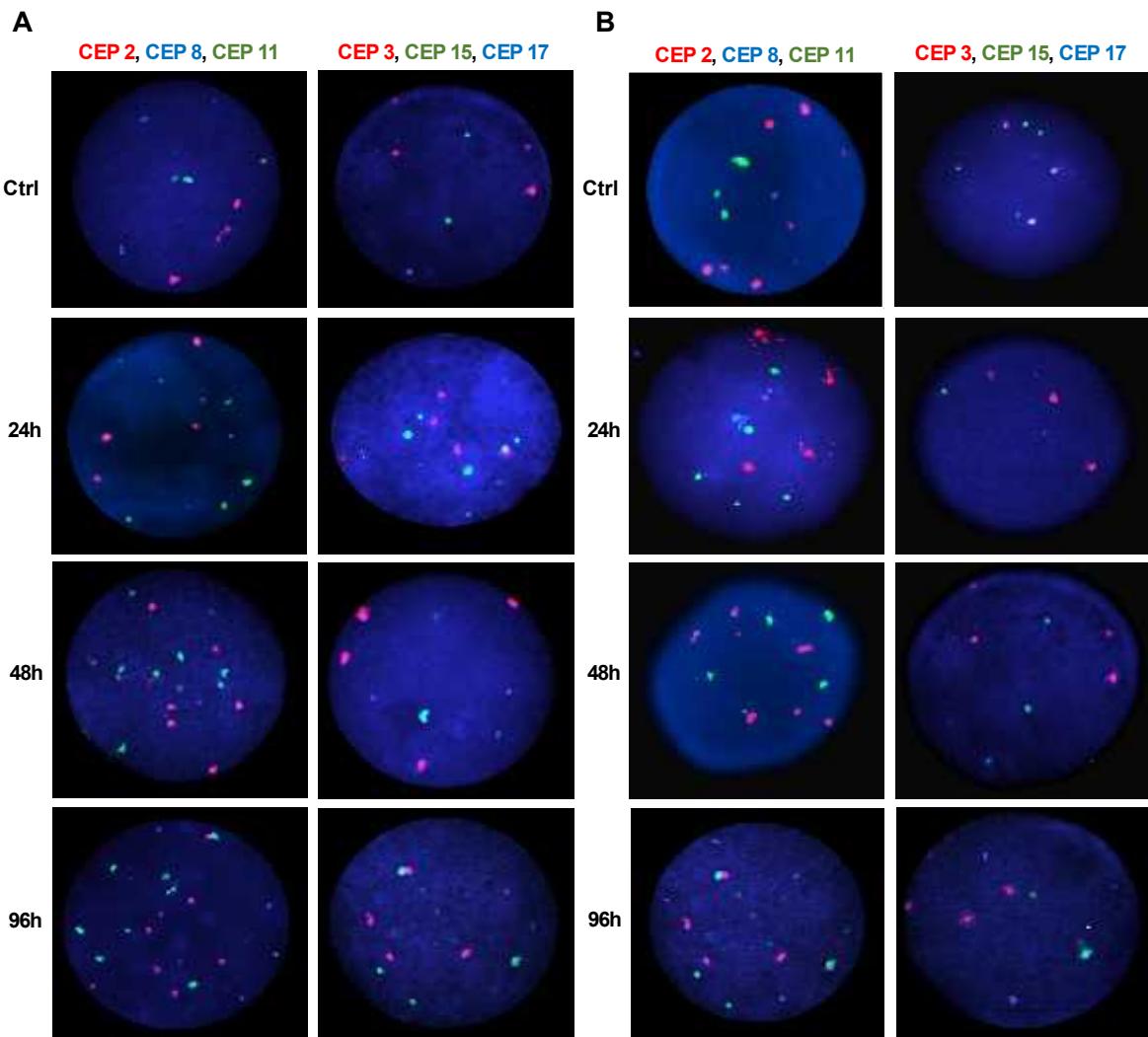


Figure 8. Representative FISH images of the MCF7 breast cancer cells after A) DOC treatment and B) TAM+DOX treatment. Three-color FISH was performed on nuclei spreads for chromosomes 2, 8 and 11 and, chromosomes 3, 15 and 17 using centromeric probes (CEP) labeled with different spectrum colors: spectrum orange for CEP2 and CEP3; spectrum green for CEP8 and CEP17; and spectrum aqua for CEP11 and CEP15. Interphase nuclei at each treatment time point are indicated. Ctrl: Control, untreated cells.

2.2.3. MDA-MB468 cells

In triple negative cells (ER β -/PR-/HER2-), all individual treatments stimulated cell proliferation (Figure 2E and Table S4) and induced an increase in CIN from 50% in untreated cells to 67% after treatments (Tables S1 and S4). However, these cells after treatments presented a relatively stable CH (low CH).

Among the individual treatments, DOX showed the poorest effects, since it not only significantly stimulated cell proliferation ($p < 0.01$, Student's t-test), but also increased CIN from 50% in untreated cells to 57% after treatment, and CH from 1.18 in untreated cells to 1.36 after treatment. Similar results were observed for the combined



treatments, in which TAM+DOC and TAM+DOX treatments significantly stimulated cell proliferation ($p < 0.02$) and increased CIN, which did not exceed 64% (Figures 3 and 5).

These results suggest that triple negative cells with intermediate CIN and low CH are resistant to chemotherapy (taxanes and anthracyclines), hormonal therapy and combined treatments (chemotherapy + hormonal treatment).

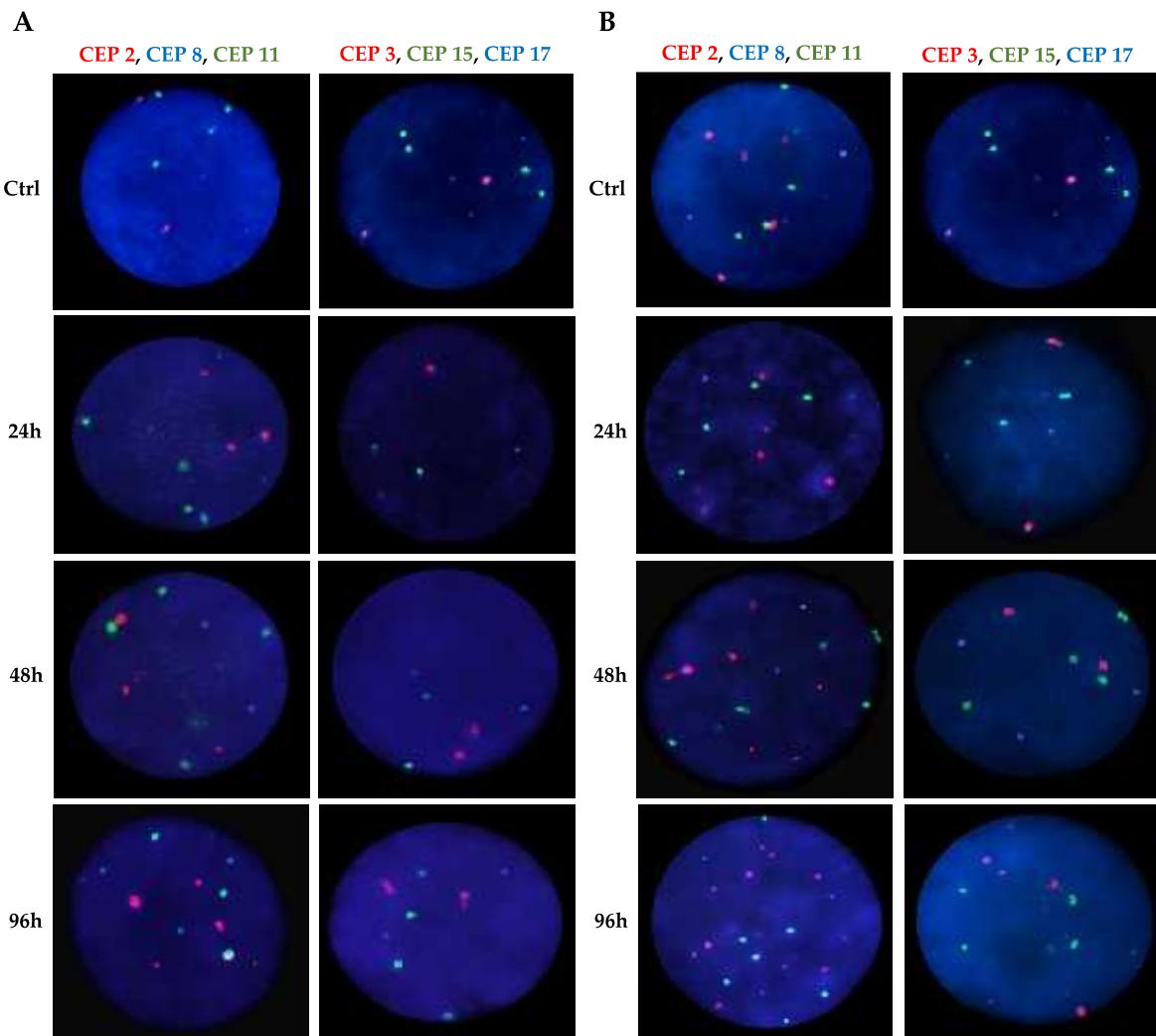


Figure 9. Representative FISH images of the MDA-MB468 breast cancer cells after A) TAM+DOC treatment and B) DOX treatment. Three-color FISH was performed on nuclei spreads for chromosomes 2, 8 and 11 and, chromosomes 3, 15 and 17 using centromeric probes (CEP) labeled with different spectrum colors: spectrum orange for CEP2 and CEP3; spectrum green for CEP8 and CEP17; and spectrum aqua for CEP11 and CEP15. Interphase nuclei at each treatment time point are indicated. Ctrl: Control, untreated cells.

2.3. Variation of CIN and CH after treatments in HER2+ cells

2.3.1. BT474 cells

In ER β +/PR+/HER2+ cells, the best effects in terms of reduction of cell proliferation were observed when the treatments applied individually or in combination did not increase the CIN above 68% (Figures 6 and 7, and Tables S1 and S5). Among the individual treatments, the treatment that showed the greatest inhibition of cell



proliferation was HT, which also increased the CIN from 61% in the untreated cells to 67% after treatment. In contrast, DOC significantly stimulated cell proliferation ($p < 0.001^{**}$, Figure 6A) mainly at 96 h and induced an increase in CIN (72%) and CH (1.7) (Figure 7 and Tables S1 and S5). However, the combination of DOC and HT was superior to the use of chemotherapy with taxane alone, in which HT+DOC treatment inhibited cell proliferation (Figure 6B) and increased CIN to 65% compared with the untreated cells (61%). Similar results were observed when DOX was used as monotherapy, where it inhibited cell proliferation and increased CIN below 68%. While the administration of DOX, combined with TAM or HT, stimulated cell proliferation and increased CIN (above 69%) and CH (between 1.6 and 1.8).

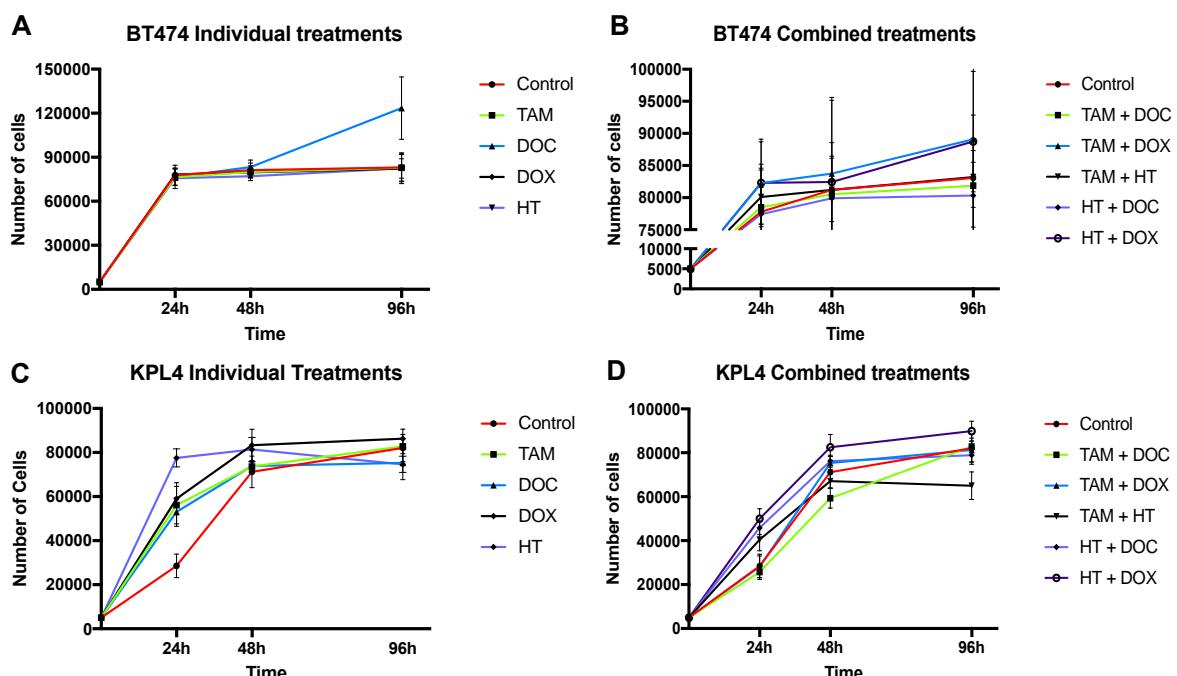


Figure 10. Effects of tamoxifen (TAM), docetaxel (DOC), doxorubicin (DOX), herceptin (HT), TAM+DOC, TAM+DOX, TAM+HT, HT+DOC and HT+DOX treatments for 24 h, 48 h, and 96 h on proliferation in (A-B) BT474 cells and (C-D) KPL4 cells. Error bars represent mean standard deviation of 24 separate experiments.

Considering that chromosome 17 remained stable throughout the treatments and no major alterations were observed in the modal number (Figure 7), we could suggest that its stability is indicative of resistance to anthracyclines, in contrast to a previous report [19], which indicated that tumor cells with CEP17 duplication (instability) may be associated with preferential sensitivity to anthracycline-based regimens.

2.3.2. KPL4 cells

In ER β -/PR-/HER2+ cells, all individual treatments showed the poorest effects, since it not only significantly stimulated cell proliferation ($p < 0.0037^{**}$, Figure 6C), but also increased CIN from 50% in untreated cells to 68% after treatments (Figure 7 and Tables S1 and S6). Contrary to what was observed in ER β +/PR+/HER2-, ER β +/PR+/HER2+ and ER β -/PR-/HER2- cells, in KPL4 cells (ER β -/PR-/HER2+) the best effects in terms of reduction of cell proliferation were observed when the treatments administered in combination increased the CIN above 70% (Figures 6D and 7, and Tables S1 and S6). Only the combined treatments between TAM with DOC,



DOX or HT, showed reduction in cell proliferation, being observed a greater reduction when TAM was combined with taxanes ($p < 0,0005^*$, Figure 6D). The administration of TAM+DOC increased both CIN, from 50% in the untreated cells to 74% after treatment (Figure S1) and CH, from 1,31 in the untreated cells to 2 after treatment (Tables S1 and S6). Whereas, HT administered as a single agent or in combination with chemotherapy (anthracyclines or taxanes) induced an increase in both cell proliferation ($p < 0,0003^*$, Figure 6B) and CIN of 50% in untreated cells to 68% after treatment.

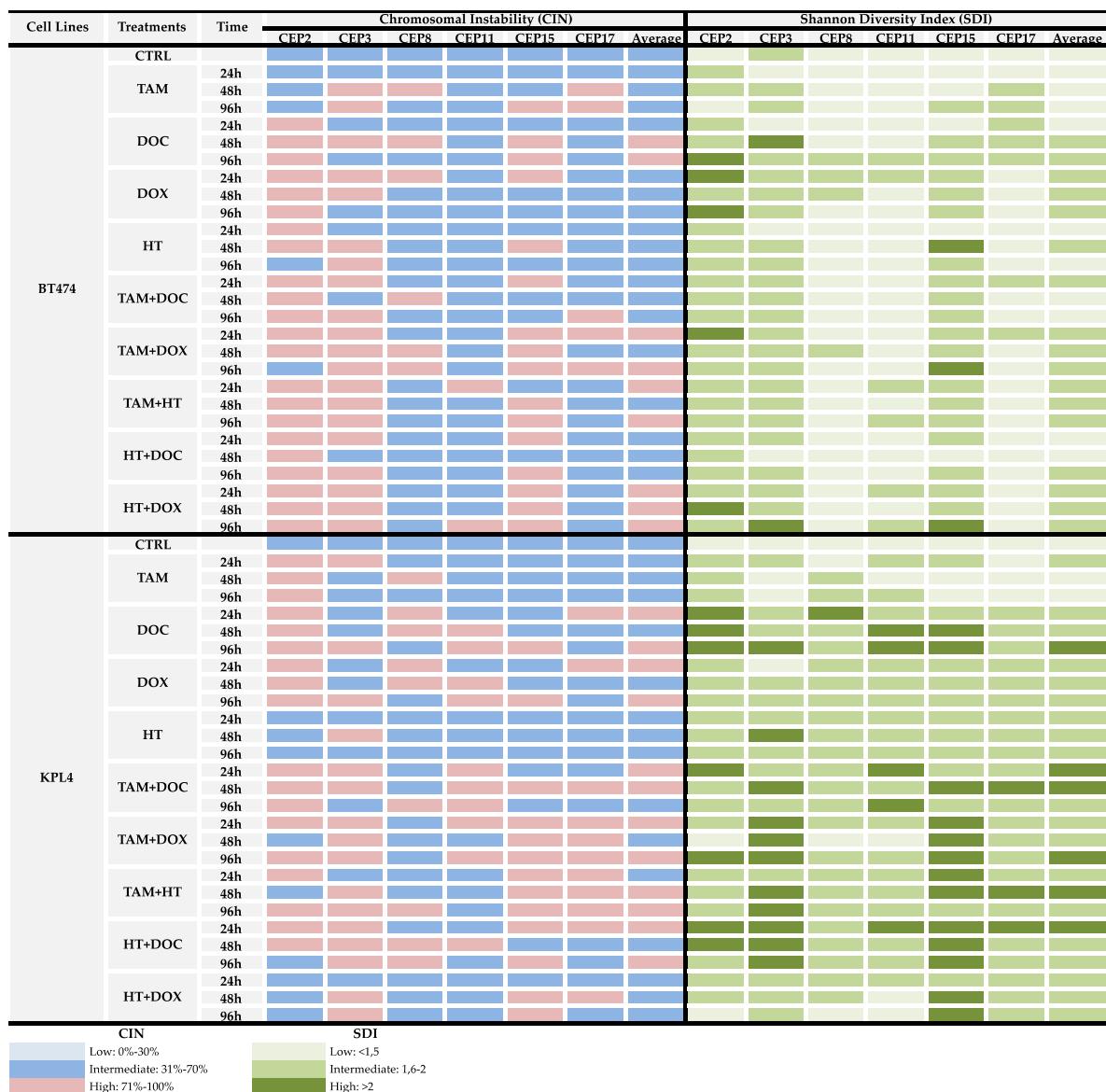


Figure 11. Chromosomal Instability (CIN) and Shannon Diversity Index (SDI) (indicative of clonal heterogeneity) induced by tamoxifen (TAM), docetaxel (DOC), doxorubicin (DOX), Herceptin (HT), TAM+DOC, TAM+DOX, TAM+HT, HT+DOC and HT+DOX in HER2+ breast cancer cells at each treatment time point. The level of CIN and the SDI before and after treatments in ER+/PR+/HER2- cells is color coded according to the legend at the bottom.

3. Discussion



BC is a heterogeneous disease in which each tumor shows individual characteristics. This has led to the search for new markers to improve not only patient diagnosis but also obtain a better response to therapy and improve prognosis. Currently, strategies for the treatment of BC depend on the tumor subtype, in which therapies are selected based on specific markers. For example, for tumors with positive hormone receptors (ER β and PR) (Luminal A and Luminal B), endocrine therapy (TAM) is applied [20,21], with some patients also requiring chemotherapy. In the case of HER2+ tumors (Luminal B and HER2+), the treatment consists of monoclonal antibodies that recognize the extracellular domain of HER2 (HT), as well as inhibitors of the tyrosine kinase domain of the HER2 receptor (such as lapatinib) [22], in addition to endocrine therapy (TAM) if the hormone receptors are also positive. For triple negative BC, only chemotherapy is applied. Chemotherapy is the only systemic therapy with proven efficacy in triple negative BC and an important complement of endocrine therapy or therapy directed to HER2 in patients with BC positive for hormone receptors (ER and PR) [23].

In this study, we observed that intermediate CIN is linked to drug sensitivity according to three characteristics, including ER β and HER2 status, the pre-existing level of CIN of the tumor cells, and the CIN induced by the treatments. In addition, all therapies used in our study (monotherapy or combined therapy) promoted CIN themselves, which influenced the response to therapy. We found that ER β +/PR+/HER2- cells with intermediate CIN were sensitive to treatment with taxanes (DOC) and anthracyclines (DOX), while ER β -/PR-/HER2- and ER β +/PR+/HER2+ cells with intermediate CIN were resistant to these drugs, mainly when anthracyclines were used in combination with TAM.

In ER β +/PR+/HER2+ cells (BT474), DOC stimulated cell proliferation and increased CIN, which exceeds 70%, while in ER β +/PR+/HER2- cells (MCF7 and ZR751 cells), DOC inhibited cell proliferation and increased CIN at 68%. Thus, in ER β +/PR+/HER2- cells, the induction of greater CIN above the pre-existing CIN (before treatment) but below 70% (after treatment) indicates sensitivity to therapy (inhibition of cell proliferation). However, in ER β +/PR+/HER2- cells, if the CIN threshold is exceeded by 70% and CH also increases, cells develop resistance to therapy. The increased rate of CIN and CH may inadvertently create a more aggressive tumor with an enhanced potential to become drug resistant.

DOC is generally recognized as one of the most effective drugs available for the treatment of metastatic BC [24] and is studied in patients who do not respond or develop resistance to chemotherapy with anthracyclines (DOX) [25,26]. However, although we observed that DOC was more effective than DOX when it was administered to ER +/PR+/HER2- tumor cells (MCF7 and ZR751) with intermediate CIN, it was not effective when administered to ER +/PR+/HER2+ cells (BT474), where DOC induced an increase in cell proliferation compared to the control.

In fact, in ER β +/PR+/HER2+ cells, intermediate CIN (61%) predicts sensitivity to treatment with TAM, DOX, HT, DOC+TAM and DOC+HT and resistance to DOC, TAM+HT, TAM+DOX and HT+DOX. Our results are consistent with those reported in breast and colorectal tumors, in which taxanes (DOC) inhibited cell proliferation when applied to tumor cells with low CIN, but induced resistance when applied to tumor cells with intermediate or high CIN [2]. Furthermore, the resistance to TAM+DOX, TAM+HT and HT+DOX observed in these cells (ER β +/PR+/HER2+ cells), with intermediate CIN and intermediate CH, could be because genetically heterogeneous tumors may harbor sub-clonal populations that exhibit different levels of CIN, resulting in cell-to-cell variations in response to treatment. CIN-inducing drugs may increase the level of CIN beyond a critical threshold and induce death for a subset of tumor cells already



exhibiting an "intermediate or high" level of CIN, whereas the viability of cells initially exhibiting a lower level of CIN may not be compromised. Together these results suggest the effectiveness of the combined use of taxanes and therapies directed against HER2 in ER β +/PR+/HER2+ cells, since these drugs may target karyotypically distinct subpopulations within the same tumor (intratumor clonal heterogeneity) [27].

In contrast to the results in BT474 cells (ER β +/PR+/HER2+), triple negative cells ER β -/PR-/HER2- with complex karyotypes (intermediate CIN=50%) and low CH (stable CIN) were resistant to all treatments used. Although all treatments increased CIN, this was possibly not enough to exceed the tolerance threshold of the cells, leading to cell survival. These results reflect the complexity of these cells, since even if the CIN increases by 2%, this increase favors cell survival and proliferation. Because we did not observe an inhibition in cell proliferation with any of the treatments, it is not possible for us to postulate a CIN threshold below or above which the treatments can induce cell survival or lethality. These results might suggest that many cancer cells demonstrate adaptation to CIN, suggesting that maintaining CIN and CH may favor cancer cell viability and survival. Thus, and as indicated by Thompson et al. (2017) [28], it is possible that triple negative cancer cells with positive CIN may exhibit intrinsic resistance to drugs that seek to increase CIN through a similar mechanism. Such intrinsic resistance can be caused by pre-existing (inherent) genetic mutations that result in decreased responsiveness of cancer cells to both chemotherapy and targeted drugs or by heterogeneity of tumors in which pre-existed insensitive subpopulations will be selected upon drug treatment, thus leading to resistance to therapeutic treatments [29].

The results observed in KPL4 cells (ER β -/PR-/HER2+), suggest that HER2 overexpression may influence the response to taxanes, where TAM significantly increase the antitumor activity of DOC, being also avoids an increase in CIN and CH. The results observed in ER -/PR-/HER2+ cells are consistent with previous studies that have shown that HER2 overexpression predicts a worse response to hormonal therapy with TAM in BC [30,31]. Although ER -/PR-/HER2+ cells are negative for ER β , the best results in terms of reduction in cell proliferation were observed when combined treatments between TAM with DOC, DOX or HT were administered to the cells. These results are not surprising since it has been reported that although ER - patients are considered non-responders, between 5% and 10% of these benefit from TAM therapy [32-34]. Such response could be due to the presence of the G protein-coupled receptor 30 (GPCR30), an estrogen transmembrane receptor, which modulates both rapid non-genomic and genomic transcriptional events of estrogen [35-37]. Of note that, similar to recent reports [38], we observe that the combination of HER2-targeted therapy (HT) with endocrine therapy (TAM) was superior to endocrine therapy alone for ER-/PR-/HER2+ cells, where a reduction in cell proliferation and an increase in CIN greater than 70% was observed. The above findings not only suggest a possible benefit of the use of these combinations as a treatment option, but also of the role of CIN in the development of cytotoxicity. Contrary to what was observed in MCF7, ZR751, MDA-MB468 and BT474 cells, in ER β -/PR-/HER2+ cells (KPL4) cells a high CIN is related to a high sensitivity to combined treatments.

In HER2+ cells (BT474 and KPL4), a greater benefit in terms of reduction of cell proliferation, was observed when the combined treatment between trastuzumab (herceptin) and chemotherapy (DOC) was administered, compared to that observed when chemotherapy was administered alone. Such benefit was also reported in an open-label randomized multicenter phase II trial (M77001), where the efficacy and safety of first-line HT with DOC compared with DOC alone in patients with HER2+ was observed [39]. Considering the above, we suggest that in cells HER2+ (BT474 y KPL4), HER2 overexpression is a predictor of resistance to taxanes.



Considering that in KPL4 cells an CIN greater than 69% was related to sensitivity or response to therapy, it could be suggested that an increase in the dose of the treatments (applied in monotherapy) could be necessary to increase CIN and to induce cytotoxicity. In fact, some studies have reported that HER2 overexpression may be a useful marker for identifying patients who are most likely to benefit from high doses of adjuvant doxorubicin-based chemotherapy. Further, previous studies demonstrated that in ER β - breast cancer, extreme CIN is associated with improved clinical outcome, which is consistent with a negative impact of CIN on tumor fitness and growth [15]. The results observed in ER β - cells (MDA-MB468 and KPL4) with intermediate CIN, suggest the need to modify the dose of the drugs to generate a higher CIN and, therefore, cellular toxicity. Therefore, defining the maximum dose at which these treatments could induce a higher CIN without side effects is required. Recently, new treatment strategies of "on and off" or "high dose followed by low dose" have been used, which result in longer survival and delayed drug resistance. This may be because intermittent or adaptive dosing may interrupt the growth of drug-dependent resistant cells and allow the competition of sensitive and resistant cells [40].

Related to resistance due to the tumor subtype, Davis and colleagues (2018) [41] reported that MCF7 cell line (ER β +/PR+/HER2-), representative of the Luminal A tumor subtype, is sensitive to DOX, while MDA-MB231 cell line (ER β -/PR-/HER2-), representative of the triple negative tumor subtype, is resistant to DOX. This report is consistent with our results, since we observed that MCF7 and ZR751 cell lines, both ER β +/PR+/HER2- and representative of the Luminal A BC subtype, are sensitive to DOX treatment, while MDA-MB468 (ER β -/PR-/HER2-) and KPL4 cell lines (ER β -/PR-/HER2+), representatives of triple negative and HER2+ BC subtype, respectively, did not respond to this drug. Moreover, the addition of DOX to MCF7 and ZR751 increased the CIN but did not exceed 67%, whereas in the KPL4 cells, DOX increased the CIN but did not exceed 69%.

Our results suggest that indeed a CIN threshold exists, which when exceeded could induce cell cytotoxicity or cell survival. For instance, in ER β +/PR+/HER2- cells (MCF7 and ZR751) and ER β +/PR+/HER2+ cells (BT474) with intermediate CIN, the therapy (chemotherapy, hormonal therapy or combined therapies) induced an increase in CIN greater than 70% and a higher CH, which correlated to cell survival. While in ER β -/PR-/HER2+ cells (KPL4) with intermediate CIN, the monotherapy and combined therapy induced an increase in CIN greater than 70% and a higher CH, which correlated to cell cytotoxicity (Figure 8).

Considering that high CH is the likely result of aneuploidy originating from CIN generating multiple unrelated clones, we speculate that both the baseline CH and CIN and those generated after drug exposure are sufficiently diverse to confer proliferative advantages and therefore resistance. Opposite results were observed in triple negative cells (ER β -/PR-/HER2-), in which monotherapy and combined treatments induced an increase in proliferation. These cells showed an increase in CIN that did not exceed 68% and a relative stability (low CH).

Consistent with a close relationship of CIN with CH in BC, there was a high association between CIN and SDI for all chromosomes and for all cell lines studied before and after treatments (Figures 3 and 7 and Table S1). These data suggest that cancer cells with high CIN (CIN>71%) have the highest SDI and thus the most extreme chromosomal numerical heterogeneity (Figures 3 and 7, and Table S1). These results are concordant with the "unstable aneuploidy" definition provided by Lingle and colleagues in an analysis of 20 breast tumors [42].

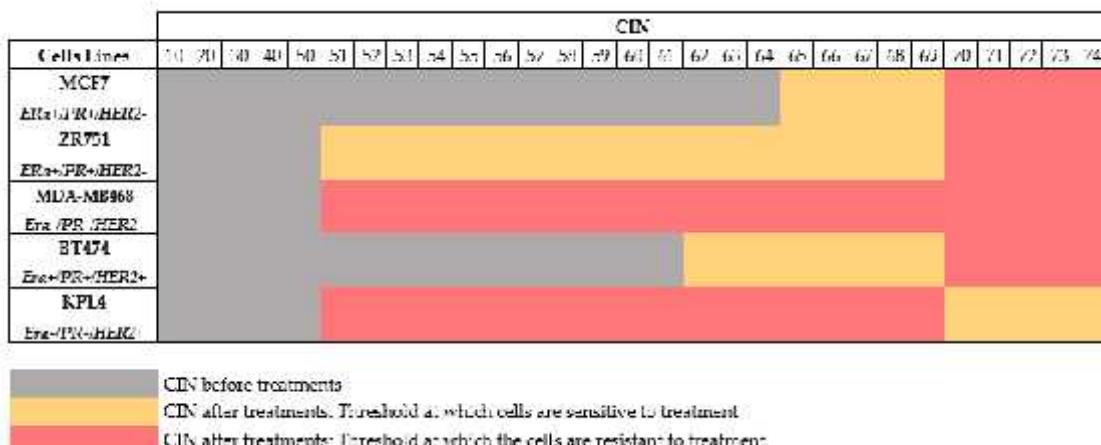


Figure 12. CIN threshold at under which cells are sensitive or resistant to treatments

The exploratory analysis of the individual chromosomes in all cell lines analyzed (MCF7, ZR751, MDA-MB468, BT474 and KPL4) highlighted chromosome (Chr) 2, Chr8 and Chr17 as candidate markers of the therapy response. Chr2 showed an unstable behavior (high CIN and high CH) after individual and combined treatments in all cell lines, in comparison with Chr8 in BT474 cells and Chr17 in MCF7, ZR751 and MDA-MB468 cells, which showed stable behavior (intermediate CIN and low CH) after treatments. In ER β +/PR+/HER2- cells, Chr17 stability is indicative of sensitivity to treatment with taxanes (DOC) and combined treatments of anthracyclines (DOX) with TAM, while in ER β -/PR-/HER2- cells, Chr17 stability is indicative of resistance to chemotherapy (DOC, DOX), hormonal therapy (TAM) and combined treatments of taxanes and anthracyclines with TAM. In ER β +/PR+/HER2+ cells, Chr8 stability is indicative of sensitivity to treatment with TAM, DOX, HT and combined treatments of taxanes (DOC) with TAM and with HT.

4. Materials and Methods

4.1. Cell lines

The human BC cell lines MCF7 and ZR75-1 (ER β +/PR+/HER2-), MDA-MB468 (ER β -/PR-/HER2-), BT474 (ER β +/PR+/HER2+) and KPL4 (ER β -/PR-/HER2+) were obtained from the American Type Culture Collection (ATCC). Cell lines were expanded and stocked at -80°C and cells obtained from these stocks were thawed and used for the experiments. To confirm the authentication of the cell lines, short tandem repeat profiles were performed at the end of experiments. MCF7, ZR75-1, BT474, KPL4 and MDA-MB468 cells were cultured in RPMI-1640 medium (Sigma, St. Louis, MO, USA), whereas BT474 cells were cultured in DMEM medium (Sigma). All culture media were supplemented with 10% fetal bovine serum (FBS) (Sigma), antibiotic-antimycotic solution (1X) (Sigma), and L-glutamine (2 mM) (Invitrogen GmbH). Cells were cultured in 75 cm^2 flasks at 37°C and 5% CO_2 . The absence of contamination with mycoplasma was confirmed by PCR assay.

4.2. Treatments



BC cell lines were treated with TAM (T5648; Sigma), DOC (sc-201436; Santa Cruz Biotechnology), DOX (sc-200923; Santa Cruz Biotechnology), HT, and combined treatments (TAM/DOC, TAM/DOX, TAM/HT, HT/DOC and HT/DOX).

TAM, DOC, DOX and HT were dissolved in absolute ethanol and diluted in media at 1 µM, 10 nM, 0,5 µM and 50 µg/ml, respectively, and then added to the culture medium for 24 h, 48 h, and 96 h. These concentrations have been demonstrated to be the lowest concentration to induce an effect on the cytoskeleton architecture in BC cells *in vitro* [43-46]. Each drug and/or combination was added to the cell lines according to expression of ER β and HER2. Specifically, cell lines positive and negative for ER β were treated with hormonal therapy (TAM) and combination of TAM with chemotherapy (DOC and DOX), while HER2-positive cell lines were treated with HT and combination of HT with chemotherapy (DOC and DOX). Untreated cells were used as controls. The treatment strategy is indicated in Table 1.

Table 1. Breast Cancer cell lines and treatment strategies

Cell line	Receptor status	Individual treatments				Combined treatments				
		TAM	DOC	DOX	HT	TAM+D OC	TAM+D OX	TAM+ HT	HT+D OC	HT+D OX
MCF7	ER+/PR+/HER 2-	X	X	X		X	X			
ZR75-1	ER+/PR+/HER 2-	X	X	X		X	X			
MDA-MB468	ER-/PR-/HER2-	X	X	X		X	X			
BT474	ER+/PR+/HER 2+	X	X	X	X	X	X	X	X	X
KPL4	ER-/PR-/HER2+	X	X	X	X	X	X	X	X	X

TAM: Tamoxifen; DOC: Docetaxel; DOX: Doxorubicin; HT: Herceptin

4.3. Proliferation assay

Cells were seeded at a density of $2.5\text{--}5 \times 10^3$ cells per 100 µL of phenol red-free medium in a 96 multi-well plate. After 24 h, cells were treated with TAM, DOC, DOX, HT and combined treatments (TAM/DOC, TAM/DOX, TAM/HT, HT/DOC and HT/DOX) for 24 h, 48 h, and 96 h. At the end of each treatment, cell proliferation was assessed using the cell proliferation ELISA kit, BrdU (Roche Diagnostics Deutschland GmbH). Measurement of absorbance was performed using a Tecan Infinite M200 reader (Tecan Trading AG, Switzerland) against a background control as blank. Each treatment was performed in 24 replicates. Data are expressed as means \pm standard deviation (S.D.).

4.4. Metaphase and nuclei spreads

To determine the induction of CIN and CH, we performed molecular cytogenetic analysis (FISH) on both control and treated BC cell lines. Metaphases were obtained using standardized harvesting protocols. Briefly, colcemid solution (0.03 µg/mL) (Sigma) was added to cultures 2.5 h before cell harvesting; cells were then treated with hypotonic solution, fixed three times with Carnoy's fixative (3:1 methanol to acetic acid), and spread on glass. Metaphase and nuclei spreads were subsequently hybridized with centromere probes using FISH.

4.5. Fluorescence *in situ* Hybridization (FISH) and CIN evaluation



CIN was evaluated on the metaphase and nuclei spreads obtained previously by FISH using six centromeric probes (CEP) for chromosomes 2, 3, 8, 11, 15 and 17 (all from Cytocell, Cambridge) and standard procedures. Three-color FISH was performed on nuclei/metaphase spreads for chromosomes 2, 8 and 11 and chromosomes 3, 15 and 17 using centromeric probes labeled with different spectrum colors: spectrum orange for CEP2 and CEP3; spectrum green for CEP8 and CEP17; and spectrum aqua for CEP11 and CEP15. Chromosomes 2 and 15 were selected as these chromosomes presented infrequent copy number alterations in a series of breast tumors analyzed by aCGH analysis [16]. Chromosomes 3, 8, 11 and 17 were selected as these chromosomes are frequently altered in BC [9]. Some studies have reported an association between alterations in chromosome 17 with sensitivity to anthracycline treatment [18]. In addition, some reports showed that the use of probes for just two chromosomes was sufficient to identify diploid from aneuploid tumors [47,48]. However, one advantage of using more than two probes is that clonal populations can be identified with greater certainty [49].

Ten randomly selected areas of each BC cell line were acquired using an Olympus microscope with the cytogenetic software Cytovision System 7.4 (Leica Biosystems Richmond, Inc.). CIN was assessed in a minimum of 100 intact and non-overlapping nuclei and some metaphases for each chromosome. The CIN rate for each cell line was defined first by calculating the percentage of nuclei with a CEP signal number different to the modal number (most common chromosome number in a tumor cell population) for each individual chromosome and then calculating the mean CIN percentage of all chromosomes analyzed [17,18]. According to the level of CIN, we classified the cell lines as having low CIN (CIN 0%–30%), intermediate CIN (CIN 31%–70%) or high CIN (CIN >70%).

Considering that the CIN may similarly classify stable aneuploid tumor cells with relatively few clones making up a large proportion of the tumor (e.g. 80% nuclei with two centromeres and 20% nuclei with three centromeres) together with unstable aneuploid tumor cells with high clone heterogeneity (e.g., 70% of nuclei with two centromeres, 20% with three, 5% with four and 5% with five centromeric signals), we calculated the SDI to assess more directly CH within each cell line before and after treatments. SDI integrates both the number and abundance of cell clones within each cell line according to published methods [16,50].

4.6. Data analysis

The CIN levels observed after treatments were determined in comparison with the control. Student's *t*-test was performed to compare cell proliferation of treated cell lines with untreated cell lines. The CH within each cell line was determined by calculating the SDI, which integrates both the number and abundance of cell clones within each cell line according to published methods [16,50]. The SDI (*H*) was estimated for chromosomes 2, 3, 8, 11, 15 and 17 using the following formula:

$$H = -\sum_i p_i \ln(p_i)$$

in which *p_i* is the frequency of centromere signal, *i* [16,50]. The normal value of this *H* index is between 0,5 and 5; values below 1,5 were considered indicative of low CH, values between 1,6 and 2 were considered indicative of intermediate CH; and values higher than 2 were considered indicative of high CH [16]. All statistical analysis were carried out using the SPSS version 21 and *p* values <0.05 were considered as statistically significant.



Studies have shown that FISH enables an estimation of CH within the tumor and allows the differentiation between CIN (aneuploid tumors with high CH) and stable aneuploidy (low CH) [42,51]. Therefore, we calculated the percentage of nuclei deviating from the modal centromeric signal for chromosomes 2, 3, 8, 11, 15 and 17 separately using established methods [42,47].

5. Conclusions

Our results demonstrate that intermediate CIN in BC tumors is of prognostic value and may be able to predict response to chemotherapy, hormonal therapy or combined therapy. We also demonstrated the existence of a CIN threshold that when is exceeded can generate resistance or sensibility to treatments, and such CIN threshold is dependent on ER β and HER status. Further testing in a cohort of BC patients is required to confirm these findings.

Tumors classified due to their underlying CIN may provide a more thorough understanding of alterations at the molecular level and potentially lead to new drug targets. A greater understanding of the role of chemotherapy, hormonal therapy or combined therapy in the induction of CIN and CH in BC together with prospective studies of CIN in patients with BC could contribute to the optimization of existing therapeutic regimens. Our results emphasize the importance of determining CIN level and CH in BC tumors to direct the most effective therapeutic strategy for an individual BC patient.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figure S1: title, Table S1: title, Video S1: title.

Author Contributions: Conceptualization and design, M.R.-L. and V.E.V.; Methodology, M.R.-L., N.V.-R. and E.P.-M.; Acquisition of data, M.R.-L., N.V.-R. and E.P.-M; Analysis and interpretation of data, M.R.-L., V.E.V., N.V.-R. and E.P.-M; Writing—original draft preparation, M.R.-L., and V.E.V.; Writing—review and editing, M.R.-L., V.E.V., E.P.-M. and N.V.-R. and E.D.; Supervision, M.R.-L., and V.E.V.

Funding: This research was funded by Universidad Pedagógica y Tecnológica de Colombia, Grant DIN 14-2017.

Acknowledgments: We thank Dr. Nelson Rangel for the excellent assistance with statistical analysis and reading of the manuscript. We thank Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.

Conflicts of Interest: The authors declare no conflict of interest

References

1. Pikor, L.; Thu, K.; Vucic, E.; Lam, W. The detection and implication of genome instability in cancer. *Cancer metastasis reviews* **2013**, *32*, 341-352, doi:10.1007/s10555-013-9429-5.
2. Burrell, R.A.; Juul, N.; Johnston, S.R.; Reis-Filho, J.S.; Szallasi, Z.; Swanton, C. Targeting chromosomal instability and tumour heterogeneity in HER2-positive breast cancer. *Journal of cellular biochemistry* **2010**, *111*, 782-790, doi:10.1002/jcb.22781.



3. Tanaka, K.; Hirota, T. Chromosomal instability: A common feature and a therapeutic target of cancer. *Biochim Biophys Acta* **2016**, *1866*, 64-75, doi:10.1016/j.bbcan.2016.06.002.
4. Geigl, J.B.; Obenauf, A.C.; Schwarzbraun, T.; Speicher, M.R. Defining 'chromosomal instability'. *Trends Genet* **2008**, *24*, 64-69, doi:10.1016/j.tig.2007.11.006.
5. Gagos, S.; Irminger-Finger, I. Chromosome instability in neoplasia: chaotic roots to continuous growth. *The international journal of biochemistry & cell biology* **2005**, *37*, 1014-1033, doi:10.1016/j.biocel.2005.01.003.
6. Thompson, S.L.; Compton, D.A. Chromosomes and cancer cells. *Chromosome research : an international journal on the molecular, supramolecular and evolutionary aspects of chromosome biology* **2011**, *19*, 433-444, doi:10.1007/s10577-010-9179-y.
7. Heng, H.H.; Bremer, S.W.; Stevens, J.B.; Horne, S.D.; Liu, G.; Abdallah, B.Y.; Ye, K.J.; Ye, C.J. Chromosomal instability (CIN): what it is and why it is crucial to cancer evolution. *Cancer metastasis reviews* **2013**, *32*, 325-340, doi:10.1007/s10555-013-9427-7.
8. Chandrakasan, S.; Ye, C.J.; Chitlur, M.; Mohamed, A.N.; Rabah, R.; Konski, A.; Heng, H.H.; Savasan, S. Malignant fibrous histiocytoma two years after autologous stem cell transplant for Hodgkin lymphoma: evidence for genomic instability. *Pediatric blood & cancer* **2011**, *56*, 1143-1145, doi:10.1002/pbc.22929.
9. Heim, S.; Mitelman, F. *Cancer Cytogenetics: Chromosomal and Molecular Genetic Aberrations of Tumor Cells*, Fourth ed.; Heim, S., Felix., M., Eds.; Wiley Blackwell: 2015.
10. Dayal, J.; Albergant, L.; Newman, T.; South, A. Quantitation of multiclarity in control and drug-treated tumour populations using high-throughput analysis of karyotypic heterogeneity. *Converg. Sci. Phys. Oncol* **2015**, *1*, doi:ttp://dx.doi.org/10.1088/2057-1739/1/2/025001.
11. Fedorenko, I.V.; Wargo, J.A.; Flaherty, K.T.; Messina, J.L.; Smalley, K.S. BRAF Inhibition Generates a Host-Tumor Niche that Mediates Therapeutic Escape. *The Journal of investigative dermatology* **2015**, *135*, 3115-3124, doi:10.1038/jid.2015.329.
12. Bartlett, J.M.; Munro, A.F.; Dunn, J.A.; McConkey, C.; Jordan, S.; Twelves, C.J.; Cameron, D.A.; Thomas, J.; Campbell, F.M.; Rea, D.W., et al. Predictive markers of anthracycline benefit: a prospectively planned analysis of the UK National Epirubicin Adjuvant Trial (NEAT/BR9601). *Lancet Oncol* **2010**, *11*, 266-274, doi:10.1016/S1470-2045(10)70006-1.
13. O'Malley, F.P.; Chia, S.; Tu, D.; Shepherd, L.E.; Levine, M.N.; Bramwell, V.H.; Andrusis, I.L.; Pritchard, K.I. Topoisomerase II alpha and responsiveness of breast cancer to adjuvant chemotherapy. *J Natl Cancer Inst* **2009**, *101*, 644-650, doi:10.1093/jnci/djp067.
14. Birkbak, N.J.; Eklund, A.C.; Li, Q.; McClelland, S.E.; Endesfelder, D.; Tan, P.; Tan, I.B.; Richardson, A.L.; Szallasi, Z.; Swanton, C. Paradoxical relationship between chromosomal instability and survival outcome in cancer. *Cancer research* **2011**, *71*, 3447-3452, doi:10.1158/0008-5472.CAN-10-3667.
15. Jamal-Hanjani, M.; A'Hern, R.; Birkbak, N.J.; Gorman, P.; Gronroos, E.; Ngang, S.; Nicola, P.; Rahman, L.; Thanopoulou, E.; Kelly, G., et al. Extreme chromosomal instability forecasts improved outcome in ER-negative breast cancer: a prospective validation cohort study from the TACT trial. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* **2015**, *26*, 1340-1346, doi:10.1093/annonc/mdv178.



16. Roylance, R.; Endesfelder, D.; Gorman, P.; Burrell, R.A.; Sander, J.; Tomlinson, I.; Hanby, A.M.; Speirs, V.; Richardson, A.L.; Birkbak, N.J., et al. Relationship of extreme chromosomal instability with long-term survival in a retrospective analysis of primary breast cancer. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* **2011**, 20, 2183-2194, doi:10.1158/1055-9965.EPI-11-0343.
17. Lengauer, C.; Kinzler, K.W.; Vogelstein, B. Genetic instability in colorectal cancers. *Nature* **1997**, 386, 623-627, doi:10.1038/386623a0.
18. Munro, A.F.; Twelves, C.; Thomas, J.S.; Cameron, D.A.; Bartlett, J.M. Chromosome instability and benefit from adjuvant anthracyclines in breast cancer. *Br J Cancer* **2012**, 107, 71-74, doi:10.1038/bjc.2012.232.
19. Burrell, R.A.; McClelland, S.E.; Endesfelder, D.; Groth, P.; Weller, M.C.; Shaikh, N.; Domingo, E.; Kanu, N.; Dewhurst, S.M.; Gronroos, E., et al. Replication stress links structural and numerical cancer chromosomal instability. *Nature* **2013**, 494, 492-496, doi:10.1038/nature11935.
20. Parisot, J.P.; Hu, X.F.; DeLuise, M.; Zalcberg, J.R. Altered expression of the IGF-1 receptor in a tamoxifen-resistant human breast cancer cell line. *Br J Cancer* **1999**, 79, 693-700, doi:10.1038/sj.bjc.6690112.
21. Berry, D.A.; Muss, H.B.; Thor, A.D.; Dressler, L.; Liu, E.T.; Broadwater, G.; Budman, D.R.; Henderson, I.C.; Barcos, M.; Hayes, D., et al. HER-2/neu and p53 expression versus tamoxifen resistance in estrogen receptor-positive, node-positive breast cancer. *J Clin Oncol* **2000**, 18, 3471-3479.
22. Tai, W.; Mahato, R.; Cheng, K. The role of HER2 in cancer therapy and targeted drug delivery. *J Control Release* **2010**, 146, 264-275, doi:10.1016/j.jconrel.2010.04.009.
23. Waks, A.G.; Winer, E.P. Breast Cancer Treatment: A Review. *JAMA* **2019**, 321, 288-300, doi:10.1001/jama.2018.19323.
24. Crown, J. A review of the efficacy and safety of docetaxel as monotherapy in metastatic breast cancer. *Semin Oncol* **1999**, 26, 5-9.
25. Crown, J. Docetaxel: overview of an active drug for breast cancer. *Oncologist* **2001**, 6 Suppl 3, 1-4.
26. Verweij, J.; Clavel, M.; Chevalier, B. Paclitaxel (Taxol) and docetaxel (Taxotere): not simply two of a kind. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* **1994**, 5, 495-505, doi:10.1093/oxfordjournals.annonc.a058903.
27. Pack, S.D.; Alper, O.M.; Stromberg, K.; Augustus, M.; Ozdemirli, M.; Miermont, A.M.; Klus, G.; Rusin, M.; Slack, R.; Hacker, N.F., et al. Simultaneous suppression of epidermal growth factor receptor and c-erbB-2 reverses aneuploidy and malignant phenotype of a human ovarian carcinoma cell line. *Cancer research* **2004**, 64, 789-794.
28. Thompson, L.L.; Jeusset, L.M.; Lepage, C.C.; McManus, K.J. Evolving Therapeutic Strategies to Exploit Chromosome Instability in Cancer. *Cancers (Basel)* **2017**, 9, doi:10.3390/cancers9110151.
29. Kelderman, S.; Schumacher, T.N.; Haanen, J.B. Acquired and intrinsic resistance in cancer immunotherapy. *Molecular oncology* **2014**, 8, 1132-1139, doi:10.1016/j.molonc.2014.07.011.
30. Wright, C.; Nicholson, S.; Angus, B.; Sainsbury, J.R.; Farndon, J.; Cairns, J.; Harris, A.L.; Horne, C.H. Relationship between c-erbB-2 protein product expression and response to endocrine therapy in advanced breast cancer. *Br J Cancer* **1992**, 65, 118-121, doi:10.1038/bjc.1992.22.



31. Carlomagno, C.; Perrone, F.; Gallo, C.; De Laurentiis, M.; Lauria, R.; Morabito, A.; Pettinato, G.; Panico, L.; D'Antonio, A.; Bianco, A.R., et al. c-erb B2 overexpression decreases the benefit of adjuvant tamoxifen in early-stage breast cancer without axillary lymph node metastases. *J Clin Oncol* **1996**, *14*, 2702-2708, doi:10.1200/JCO.1996.14.10.2702.
32. Early Breast Cancer Trialists' Collaborative Group; Davies, C.; Godwin, J.; Gray, R.; Clarke, M.; Cutter, D.; Darby, S.; McGale, P.; Pan, H.C.; Taylor, C., et al. Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. *Lancet* **2011**, *378*, 771-784, doi:10.1016/S0140-6736(11)60993-8.
33. Early Breast Cancer Trialists' Collaborative Group. Tamoxifen for early breast cancer: an overview of the randomised trials. . *Lancet* **1998**, *351*, 1451-1467.
34. Early Breast Cancer Trialists' Collaborative Group. Systemic treatment of early breast cancer by hormonal, cytotoxic, or immune therapy. 133 randomised trials involving 31,000 recurrences and 24,000 deaths among 75,000 women. . *Lancet* **1992**, *339*, 71-85.
35. Thomas, P.; Pang, Y.; Filardo, E.J.; Dong, J. Identity of an estrogen membrane receptor coupled to a G protein in human breast cancer cells. *Endocrinology* **2005**, *146*, 624-632, doi:10.1210/en.2004-1064.
36. Chen, J.Q.; Russo, J. ERalpha-negative and triple negative breast cancer: molecular features and potential therapeutic approaches. *Biochim Biophys Acta* **2009**, *1796*, 162-175, doi:10.1016/j.bbcan.2009.06.003.
37. Cheng, S.B.; Graeber, C.T.; Quinn, J.A.; Filardo, E.J. Retrograde transport of the transmembrane estrogen receptor, G-protein-coupled-receptor-30 (GPR30/GPER) from the plasma membrane towards the nucleus. *Steroids* **2011**, *76*, 892-896, doi:10.1016/j.steroids.2011.02.018.
38. Ruta Rao, M.C. HER2-Positive Breast Cancer. In *HER2-Positive Breast Cancer*, Elsevier, Ed. Elsevier: St. Louis, Missouri 63043, 2019; <https://doi.org/10.1016/B978-0-323-58122-6.12001-X>.
39. Marty, M.; Cognetti, F.; Maraninchi, D.; Snyder, R.; Mauriac, L.; Tubiana-Hulin, M.; Chan, S.; Grimes, D.; Anton, A.; Lluch, A., et al. Randomized phase II trial of the efficacy and safety of trastuzumab combined with docetaxel in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer administered as first-line treatment: the M77001 study group. *J Clin Oncol* **2005**, *23*, 4265-4274, doi:10.1200/JCO.2005.04.173.
40. Kaiser, J. When less is more. *Science* **2017**, *355*, 1144-1146, doi:10.1126/science.355.6330.1144.
41. Davis, T.; van Niekerk, G.; Peres, J.; Prince, S.; Loos, B.; Engelbrecht, A.M. Doxorubicin resistance in breast cancer: A novel role for the human protein AHNAK. *Biochem Pharmacol* **2018**, *148*, 174-183, doi:10.1016/j.bcp.2018.01.012.
42. Lingle, W.L.; Barrett, S.L.; Negron, V.C.; D'Assoro, A.B.; Boeneman, K.; Liu, W.; Whitehead, C.M.; Reynolds, C.; Salisbury, J.L. Centrosome amplification drives chromosomal instability in breast tumor development. *Proceedings of the National Academy of Sciences of the United States of America* **2002**, *99*, 1978-1983, doi:10.1073/pnas.032479999.
43. Sapino, A.; Pietribiasi, F.; Bussolati, G.; Marchisio, P.C. Estrogen- and tamoxifen-induced rearrangement of cytoskeletal and adhesion structures in breast cancer MCF-7 cells. *Cancer research* **1986**, *46*, 2526-2531.
44. Hartmann, K.; Becker-Putsche, M.; Bocklitz, T.; Pachmann, K.; Niendorf, A.; Rosch, P.; Popp, J. A study of Docetaxel-induced effects in MCF-7 cells by



- means of Raman microspectroscopy. *Anal Bioanal Chem* **2012**, *403*, 745-753, doi:10.1007/s00216-012-5887-9.
45. Wang, S.; Konorev, E.A.; Kotamraju, S.; Joseph, J.; Kalivendi, S.; Kalyanaraman, B. Doxorubicin induces apoptosis in normal and tumor cells via distinctly different mechanisms. intermediacy of H(2)O(2)- and p53-dependent pathways. *J Biol Chem* **2004**, *279*, 25535-25543, doi:10.1074/jbc.M400944200.
46. Ginestier, C.; Adelaide, J.; Goncalves, A.; Repellini, L.; Sircoulomb, F.; Letessier, A.; Finetti, P.; Geneix, J.; Charafe-Jauffret, E.; Bertucci, F., et al. ERBB2 phosphorylation and trastuzumab sensitivity of breast cancer cell lines. *Oncogene* **2007**, *26*, 7163-7169, doi:10.1038/sj.onc.1210528.
47. Fiegl, M.; Kaufmann, H.; Zojer, N.; Schuster, R.; Wiener, H.; Mullauer, L.; Roka, S.; Huber, H.; Drach, J. Malignant cell detection by fluorescence in situ hybridization (FISH) in effusions from patients with carcinoma. *Hum Pathol* **2000**, *31*, 448-455.
48. Takami, S.; Kawasome, C.; Kinoshita, M.; Koyama, H.; Noguchi, S. Chromosomal instability detected by fluorescence in situ hybridization in Japanese breast cancer patients. *Clin Chim Acta* **2001**, *308*, 127-131.
49. Farabegoli, F.; Santini, D.; Ceccarelli, C.; Taffurelli, M.; Marrano, D.; Baldini, N. Clone heterogeneity in diploid and aneuploid breast carcinomas as detected by FISH. *Cytometry* **2001**, *46*, 50-56.
50. Maley, C.C.; Galipeau, P.C.; Finley, J.C.; Wongsurawat, V.J.; Li, X.; Sanchez, C.A.; Paulson, T.G.; Blount, P.L.; Risques, R.A.; Rabinovitch, P.S., et al. Genetic clonal diversity predicts progression to esophageal adenocarcinoma. *Nature genetics* **2006**, *38*, 468-473, doi:10.1038/ng1768.
51. Chin, K.; DeVries, S.; Fridlyand, J.; Spellman, P.T.; Roydasgupta, R.; Kuo, W.L.; Lapuk, A.; Neve, R.M.; Qian, Z.; Ryder, T., et al. Genomic and transcriptional aberrations linked to breast cancer pathophysiology. *Cancer cell* **2006**, *10*, 529-541, doi:10.1016/j.ccr.2006.10.009.



© 2019 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).



CONCLUSIONES GENERALES

El Cáncer de seno es una enfermedad común y representa uno de los mayores problemas de salud en Colombia. Uno de los principales problemas clínicos significantes en el tratamiento de pacientes con CS radica en el desarrollo de resistencia a la terapia, por lo que los esfuerzos por elucidar tanto los mecanismos moleculares implicados en tal resistencia como el establecimiento de nuevos blancos terapéuticos están bien justificados y se convierten en una necesidad. Un blanco terapéutico potencial lo constituye la determinación de la Inestabilidad cromosómica (IC), ya que esta ha sido asociadas con progresión del cáncer, aumentada invasividad y respuesta a la terapia.

Nuestros resultados sugieren que la IC intermedia en líneas celulares de CS es de valor pronostico y puede predecir la respuesta a la terapia, por lo que proponemos la existencia de un umbral de IC que depende del estado de RE y HER2. Además los tumores clasificados debido a su IC pueden proporcionar una mayor comprensión de las alteraciones a nivel molecular y podrían conducir a nuevos blancos terapéuticos.

Nuestros resultados demuestran que los tratamientos actualmente usados en CS inducen por sí mismos IC y enfatizan la importancia de determinar el nivel de IC y heterogeneidad clonal en CS para dirigir la estrategia terapéutica más efectiva.

Una mayor comprensión del papel de la quimioterapia, la terapia hormonal o la terapia combinada en la inducción de IC y HC en CS, junto con estudios prospectivos en pacientes podría contribuir a la optimización de los regímenes terapéuticos existentes y a la disminución de efectos secundarios.



RECOMENDACIONES

Se requiere realizar la evaluación de la IC y la HC en un una cohorte de pacientes con CS, lo que permita confirmar los hallazgos observados en líneas celulares y de esta manera postular la IC como un blanco terapéutico útil en el desarrollo de nuevas estrategias terapéuticas personalizadas y dirigidas a blanquear tumores con diversos niveles de IC.



ANEXOS

Anexo 1. Chromosomal Instability (CIN) and Shannon Diversity Index (SDI) for all cell lines before and after treatments at 24 h, 48 h and 96 h

Cell Line	Treatment	Time	Chromosomal Instability (CIN)						Average CIN	Shannon Diversity Index (SDI)						Average SDI
			CE P2	CEP 3	CEP 8	CEP 11	CEP 15	CEP 17		CEP 2	CEP 3	CEP 8	CEP 11	CEP 15	CEP 17	
MCF7 ER+/PR+/HER2-	CTRL		53	68	65	62	74	63	64	1,38	1,53	1,56	1,43	1,63	1,48	1,5
	TAM	24h	60	75	52	62	72	67	65	1,49	1,68	1,12	1,22	1,6	1,38	1,41
		48h	56	52	56	70	62	67	61	1,3	1,29	1,08	1,53	1,5	1,5	1,36
		96h	74	62	65	68	50	53	62	1,84	1,35	1,45	1,55	1,14	1,18	1,41
	DOC	24h	79	57	64	73	65	60	66	2,15	1,64	1,54	1,93	1,54	1,29	1,68
		48h	79	67	70	62	66	62	68	2,02	1,67	1,54	1,8	1,54	1,3	1,64
		96h	76	70	71	65	68	59	68	1,81	1,75	1,6	1,68	1,66	1,23	1,62
	DOX	24h	75	63	60	74	68	62	67	1,88	1,36	1,4	1,77	1,58	1,25	1,54
		48h	75	64	60	58	66	63	64	1,84	1,47	1,33	1,6	1,6	1,36	1,53
		96h	72	57	57	61	58	62	61	1,8	1,51	1,33	1,48	1,54	1,36	1,5
	TAM+DOC	24h	73	75	70	71	73	68	72	2	1,86	1,57	2	1,76	1,45	1,74
		48h	48	74	63	59	66	70	63	1,19	1,8	1,4	1,41	1,61	1,42	1,47
		96h	80	69	71	81	49	59	68	2	1,76	1,7	2	1,52	1,33	1,71
	TAM+DOX	24h	75	63	56	58	62	58	62	1,81	1,53	1,34	1,5	1,43	1,22	1,47
		48h	59	56	60	68	74	63	63	1,68	1,47	1,47	1,65	1,71	1,34	1,55
		96h	76	44	57	64	41	57	57	1,7	1,36	1,31	1,61	1,19	1,33	1,41
ZR751 ER+/PR+/HER2-	CTRL		50	45	56	46	58	47	50	1,19	1,24	1,13	1,14	1,32	1,16	1,2
	TAM	24h	62	46	45	61	58	43	53	1,4	1,15	1,07	1,43	1,47	1,45	1,33
		48h	47	49	49	59	64	53	54	1,34	1,16	1,19	1,51	1,41	1,22	1,3
		96h	58	69	45	61	63	55	59	1,41	1,61	1,19	1,52	1,5	1,25	1,41
	DOC	24h	69	72	63	62	64	69	67	1,84	1,75	1,35	1,41	1,7	1,6	1,61
		48h	70	52	66	61	62	53	61	1,63	1,32	1,54	1,55	1,48	1,3	1,47



		96h	68	66	60	63	61	56	62	1,61	1,63	1,03	1,55	1,62	1,46	1,48
DOX	24h	64	49	48	45	48	57	52	1,42	1,36	1,9	1,17	1,25	1,29	1,4	
	48h	59	44	66	67	61	50	58	1,45	1,15	1,39	1,63	1,47	1,22	1,39	
	96h	68	48	72	55	50	65	60	1,85	1,23	1,72	1,55	1,13	1,36	1,47	
TAM+DO C	24h	68	55	59	47	62	59	58	1,6	1,42	1,3	1,33	1,3	1,3	1,38	
	48h	78	71	65	50	65	73	67	2,02	2,03	1,58	1,4	1,83	1,81	1,78	
	96h	69	63	73	62	64	69	67	1,65	1,76	1,55	1,41	1,62	1,59	1,6	
TAM+DO X	24h	69	76	71	68	72	62	70	1,76	1,85	1,67	1,7	1,8	1,46	1,7	
	48h	74	60	67	57	65	60	64	1,88	1,33	1,67	1,51	1,56	1,36	1,55	
	96h	74	63	62	62	67	61	65	2	1,66	1,27	1,55	1,65	1,31	1,57	
MDA-MB468 ER-/PR-/HER2-	CTRL		75	48	58	30	33	54	50	1,91	1,2	1,22	0,82	0,9	1,05	1,18
	TAM	24h	79	78	65	32	73	63	65	2,03	2	1,54	1,04	1,75	1,32	1,61
		48h	75	68	55	45	61	61	61	2	1,56	1,17	1,27	1,5	1,41	1,47
		96h	71	57	50	31	53	61	54	1,65	1,37	1,08	0,96	1,22	1,33	1,27
	DOC	24h	69	79	66	54	49	64	64	1,73	2,06	1,48	1,35	1,32	1,31	1,54
		48h	68	67	57	44	45	56	56	2	1,54	1,31	1,28	1,18	1,35	1,43
		96h	81	69	67	65	49	70	67	2,01	1,6	1,37	1,6	1,27	1,53	1,56
	DOX	24h	74	56	63	31	44	45	52	1,76	1,18	1,31	1,08	1,05	1,08	1,24
		48h	62	60	64	39	40	53	53	1,63	1,18	1,37	1,16	0,9	1,19	1,24
		96h	74	46	68	58	40	60	58	1,76	1,18	1,46	1,56	1,03	1,16	1,36
	TAM+DO C	24h	62	55	61	42	47	54	54	1,54	1,18	1,38	1,18	1,11	1,26	1,28
		48h	80	62	65	34	32	63	56	2,09	1,38	1,37	0,97	0,91	1,28	1,18
		96h	77	79	57	34	69	68	64	2	2	1,38	0,99	1,62	1,44	1,55
	TAM+DO X	24h	80	64	62	45	30	61	57	2	1,28	1,33	1,46	1	1,28	1,4
		48h	70	51	57	36	27	52	48	1,83	1,06	1,29	1,16	0,8	1,14	1,21
		96h	78	24	58	30	24	56	45	1,87	1,14	1,28	0,93	0,74	1,3	1,21
BT474	CTRL		62	69	42	66	60	65	61	1,33	1,6	0,95	1,46	1,45	1,57	1,4



ER+/PR+/HER2+	TAM	24h	67	63	69	54	60	49	61	1,6	1,48	1,55	1,35	1,32	1,05	1,4
		48h	68	74	71	57	59	73	67	1,63	1,65	1,58	1,41	1,51	1,63	1,56
		96h	63	72	69	57	75	71	68	1,5	1,64	1,37	1,35	1,81	1,6	1,55
	DOC	24h	75	70	69	59	65	73	68	1,9	1,48	1,55	1,44	1,31	1,79	1,57
		48h	73	80	72	64	74	72	72	1,92	2,23	1,27	1,5	1,87	1,74	1,75
		96h	81	70	70	63	73	69	72	2,28	1,79	1,79	1,67	1,81	1,74	1,85
	DOX	24h	81	73	76	65	71	58	71	2,34	1,86	1,82	1,66	1,81	1,37	1,81
		48h	74	71	69	46	67	67	66	1,9	1,69	1,61	1,2	1,67	1,46	1,6
		96h	78	68	66	63	61	68	67	2,08	1,67	1,51	1,58	1,6	1,52	1,66
	HT	24h	71	55	67	48	65	61	61	1,78	1,31	1,51	1,28	1,51	1,32	1,45
		48h	73	70	61	66	76	57	67	1,85	1,79	1,28	1,5	2,01	1,39	1,64
		96h	68	70	63	58	69	67	66	1,74	1,69	1,53	1,37	1,64	1,42	1,57
	TAM+DO C	24h	73	71	69	63	71	70	70	1,74	1,63	1,48	1,39	1,82	1,64	1,62
		48h	72	66	71	46	68	61	64	1,79	1,68	1,57	1,2	1,63	1,5	1,56
		96h	73	70	53	49	68	71	64	1,84	1,71	1,33	1,35	1,69	1,46	1,56
	TAM+DO X	24h	80	76	69	53	81	73	72	2,11	1,95	1,51	1,51	1,99	1,65	1,79
		48h	73	71	73	56	75	68	69	1,79	1,78	1,68	1,48	1,69	1,4	1,64
		96h	69	78	70	53	83	72	71	1,83	1,9	1,53	1,34	2,1	1,5	1,7
	TAM+HT	24h	78	78	63	76	64	64	71	1,87	1,99	1,55	1,88	1,84	1,46	1,77
		48h	74	74	60	62	74	67	69	1,77	1,81	1,48	1,49	1,78	1,44	1,63
		96h	71	76	69	70	79	64	72	1,78	1,86	1,48	1,62	1,99	1,47	1,7
	HT+DOC	24h	71	73	50	61	78	59	65	1,79	1,66	1,2	1,19	1,85	1,54	1,54
		48h	76	68	65	59	64	54	64	1,81	1,56	1,28	1,52	1,5	1,37	1,51
		96h	79	74	59	52	71	57	65	1,99	1,85	1,29	1,32	1,82	1,44	1,62
	HT+DOX	24h	73	76	65	69	76	64	71	1,74	1,9	1,34	1,6	2	1,48	1,68
		48h	79	78	63	65	76	64	71	2,2	1,9	1,49	1,47	1,92	1,36	1,72
		96h	75	75	66	74	78	65	72	2	2,03	1,36	1,84	2,05	1,48	1,79
KPL4	CTRL		48	57	43	53	57	45	51	1,18	1,37	1,27	1,3	1,5	1,24	1,31



ER-/PR-/HER2+	TAM	24h	76	71	64	68	66	63	68	1,93	1,77	1,51	1,62	1,71	1,52	1,68
		48h	79	54	76	64	50	66	65	1,94	1,09	1,79	1,55	1,24	1,46	1,51
		96h	71	52	69	70	48	62	62	1,75	1,14	1,7	1,67	0,95	1,38	1,43
	DOC	24h	75	68	79	63	69	74	71	2,1	1,9	2	1,77	1,81	1,9	1,91
		48h	74	64	71	73	66	66	69	2,02	1,82	1,77	2	2	1,76	1,86
		96h	74	79	65	71	72	70	72	2,03	2,1	1,71	2	2,02	1,84	2
	DOX	24h	75	68	79	63	69	74	71	1,74	1,53	1,7	1,73	1,7	1,73	1,7
		48h	74	64	71	73	66	66	69	1,85	1,93	1,78	1,83	1,99	1,81	1,87
		96h	74	79	65	71	72	70	72	1,94	1,75	1,73	1,84	1,96	1,91	1,86
	HT	24h	67	70	64	68	65	64	66	1,61	1,75	1,74	1,66	1,76	1,6	1,69
		48h	66	74	66	69	62	70	68	1,63	2,09	1,63	1,72	1,89	1,75	1,79
		96h	67	67	66	66	67	68	67	1,81	1,66	1,6	1,65	1,78	1,73	1,7
	TAM+DO C	24h	74	1,91	76	2,1	64	1,94	72	1,96	75	2	75	1,87	73	1,96
		48h	39	1,07	85	2,53	66	1,44	63	1,52	83	2,46	75	1,87	69	1,81
		96h	74	2,03	83	2,43	65	1,71	71	1,89	80	2,33	79	1,97	75	2,06
	TAM+DO X	24h	74	1,91	76	2,1	64	1,94	72	1,96	75	2	75	1,87	73	1,96
		48h	39	1,07	85	2,53	66	1,44	63	1,52	83	2,46	75	1,87	69	1,81
		96h	74	2,03	83	2,43	65	1,71	71	1,89	80	2,33	79	1,97	75	2,06
	TAM+HT	24h	76	1,9	70	1,95	60	1,62	62	1,6	75	2,03	73	1,82	69	1,82
		48h	64	1,63	89	2,8	64	1,86	70	2	83	2,62	84	2,6	76	2,25
		96h	75	1,83	86	2,5	73	1,66	69	1,78	73	2	73	2	75	1,96
	HT+DOC	24h	71	2,01	73	2,18	66	1,83	69	2,05	74	2,23	74	2,21	71	2,09
		48h	76	2,16	72	2,08	72	1,82	73	2	63	2,01	63	1,87	69	2
		96h	70	1,73	79	2,32	71	1,65	62	1,76	75	2,33	68	1,61	71	2
	HT+DOX	24h	61	1,82	70	2	59	1,6	61	1,65	67	1,83	67	1,87	64	1,8
		48h	67	1,74	71	1,77	58	1,62	65	1,43	72	2,14	72	1,73	68	1,74
		96h	65	1,5	74	2	61	1,42	63	1,49	77	2,1	65	1,91	68	1,74



Anexo 2: Chromosomal Instability (CIN) and cell proliferation in MCF7 cells before and after treatments at 24 h, 48 h and 96 h

	MN	%CIN	Number of cells		p
			Control	Treated	
Control	4	64%	5000	5000	1
TAM. 24h	4	65%	45740	45006	0,6
TAM. 48h	4	61%	67452	66516	0,776
TAM. 96h	3	62%	55681	55172	0,872
DOC. 24h	4	66%	45740	45045	0,611
DOC. 48h	5	68%	67452	62322	0,675
DOC. 96h	4	68%	55681	52802	0,37
DOX. 24h	4	67%	45740	44275	0,306
DOX. 48h	5	64%	67452	65773	0,599
DOX. 96h	4	61%	55681	54751	0,741
TAM+DOC. 24h	5	72%	45740	49029	0,012**
TAM+DOC. 48h	4	63%	67452	65843	0,621
TAM+DOC. 96h	5	68%	55681	54341	0,658
TAM+DOX. 24h	4	62%	45740	45502	0,877
TAM+DOX. 48h	4	63%	67452	63684	0,239
TAM+DOX. 96h	4	57%	55681	51433	0,229

MN: Modal Number; p: Student's *t*-test. **p* < 0,05; ** *p* < 0,01



Anexo 3: Chromosomal Instability (CIN) and cell proliferation in ZR751 cells before and after treatments at 24 h, 48 h and 96 h

	MN	%CI N	Number of cells		p
			Control	Treated	
Control	3	50%	5000	5000	1
TAM. 24h	3	53%	11075	10761	0,33
TAM. 48h	3	54%	10224	10124	0,83
TAM. 96h	3	59%	11000	10777	0,83
DOC. 24h	3	67%	11075	10938	0,7
DOC. 48h	3	61%	10224	9985	0,66
DOC. 96h	3	62%	11000	10453	0,52
DOX. 24h	3	52%	11075	11044	0,93
DOX. 48h	3	58%	10224	10145	0,82 9
DOX. 96h	3	60%	11000	10872	0,88
TAM+DOC. 24h	3	58%	11075	10988	0,83
TAM+DOC. 48h	4	67%	10224	10136	0,81
TAM+DOC. 96h	3	67%	11000	10741	0,77
TAM+DOX. 24h	3	70%	11075	11563	0,22
TAM+DOX. 48h	3	64%	10224	10196	0,93 7
TAM+DOX. 96h	3	65%	11000	10493	0,57

MN: Modal Number; p: Student's *t*-test. **p* < 0,05; ** *p* < 0,01



Anexo 4: Chromosomal Instability (CIN) and cell proliferation in MDA-MB468 cells before and after treatments at 24 h, 48 h and 96 h

	MN	%CIN	Number of cells		p
			Control	Treated	
Control	3	50%	5000	5000	1
TAM. 24h	4	65%	17096	17625	0,49
TAM. 48h	4	61%	29398	30467	0,539
TAM. 96h	3	54%	52752	54846	0,1302
DOC. 24h	4	64%	17096	18873	0,021*
DOC. 48h	3	56%	29398	30887	0,3855
DOC. 96h	3	67%	52752	53246	0,7032
DOX. 24h	3	52%	17096	20006	0,0006*
DOX. 48h	3	53%	29398	32187	0,087
DOX. 96h	3	58%	52752	56414	0,017**
TAM+DOC. 24h	3	54%	17096	19360	0,0007**
TAM+DOC. 48h	4	56%	29398	33286	0,014**
TAM+DOC. 96h	5	64%	52752	56208	0,022*
TAM+DOX. 24h	3	57%	17096	17110	0,97
TAM+DOX. 48h	3	52%	29398	30808	0,4297
TAM+DOX. 96h	3	50%	52752	56779	0,01**

MN: Modal Number; p: Student's *t*-test. **p* < 0,05; ** *p* < 0,01



Anexo 5: Chromosomal Instability (CIN) and cell proliferation in BT474 cells before and after treatments at 24 h, 48 h and 96 h

	MN	%CI N	Number of cells		p
			Control	Treated	
Control	4	61%	5000	5000	1
TAM. 24h	4	61%	77750	76300	0,534
TAM. 48h	5	67%	81200	79950	0,502
TAM. 96h	5	68%	83000	82500	0,955
DOC. 24h	5	68%	77750	76450	0,533
DOC. 48h	6	72%	81200	83250	0,345
DOC. 96h	7	72%	83000	123000	<0,0000005*
DOX. 24h	6	71%	77750	78150	0,856
DOX. 48h	5	66%	81200	79700	0,399
DOX. 96h	4	67%	83000	82000	0,857
HT. 24h	5	61%	77750	75650	0,423
HT. 48h	5	67%	81200	77100	0,022*
HT. 96h	5	66%	83000	82000	0,868
TAM+DOC. 24h	5	70%	77750	78500	0,778
TAM+DOC. 48h	4	64%	81200	80500	0,771
TAM+DOC. 96h	5	64%	83000	81850	0,746
TAM+DOX. 24h	5	72%	77750	82250	0,097
TAM+DOX. 48h	5	69%	81200	83750	0,493
TAM+DOX. 96h	5	71%	83000	89000	0,138
TAM+HT. 24h	5	71%	77750	80100	0,304
TAM+HT. 48h	5	69%	81200	81200	0,992
TAM+HT. 96h	5	72%	83000	83200	0,966
HT+DOC. 24h	5	65%	77750	78750	0,879
HT+DOC. 48h	5	64%	81200	79850	0,638
HT+DOC. 96h	5	65%	83000	80000	0,375
HT+DOX. 24h	5	71%	77750	81250	0,086
HT+DOX. 48h	5	71%	81200	82400	0,756
HT+DOX. 96h	5	72%	83000	88500	0,21

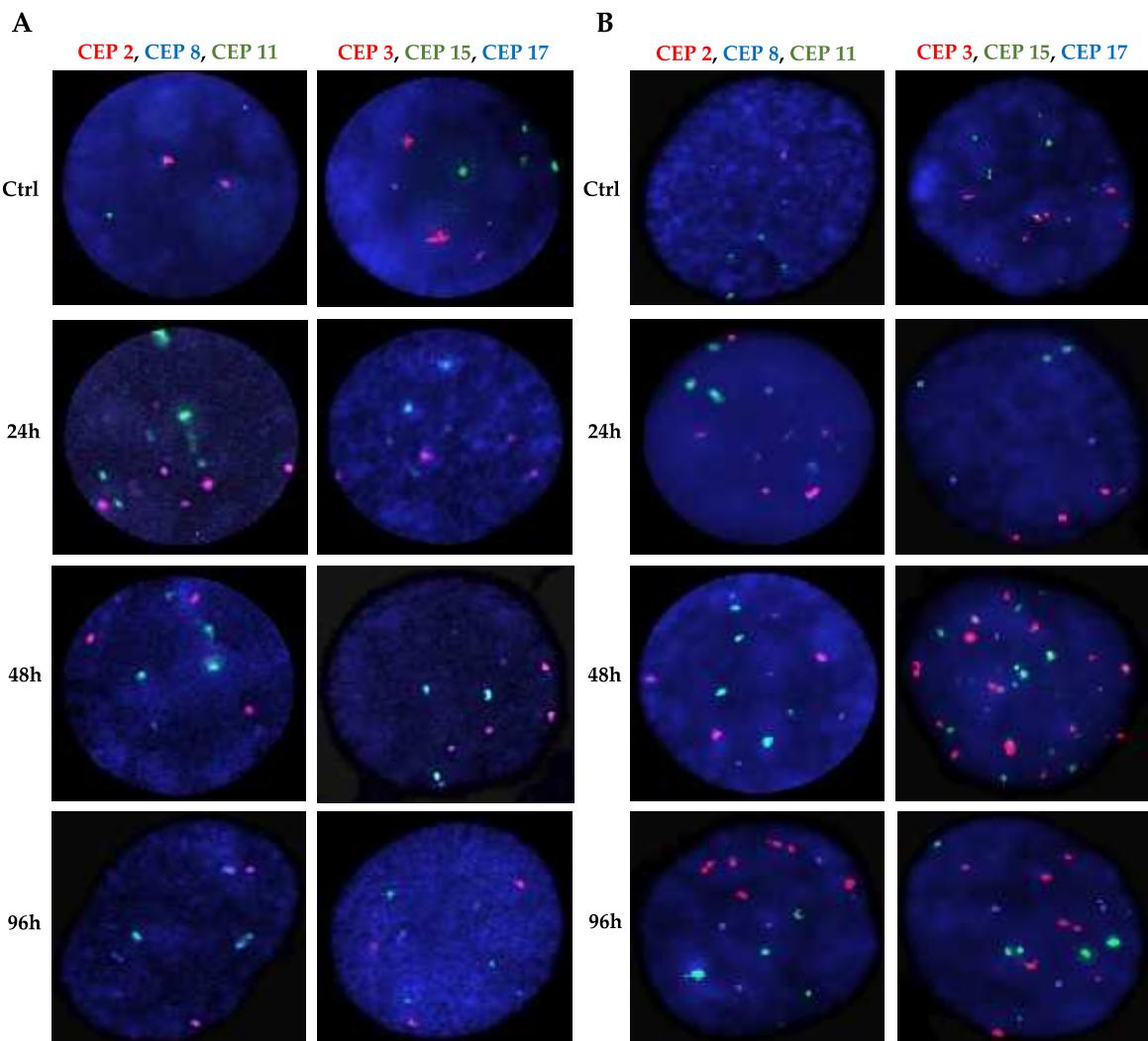
MN: Modal Number; p: Student's t-test. * $p < 0,05$; ** $p < 0,01$



Anexo 6: Chromosomal Instability (CIN) and cell proliferation in KPL4 cells before and after treatments at 24 h, 48 h and 96 h

	MN	%CIN	Number of cells		p
			Control	Treated	
Control	2	51%	5000	5000	
TAM. 24h	3	68%	28506	56118	<0,0001**
TAM. 48h	3	65%	71181	73608	0,612
TAM. 96h	3	62%	81597	82766	0,745
DOC. 24h	4	71%	28506	53038	<0,0001**
DOC. 48h	3	69%	71181	73893	0,314
DOC. 96h	3	72%	81597	74762	0,021*
DOX. 24h	3	64%	28506	59032	<0,0001**
DOX. 48h	3	69%	71181	83284	0,0007**
DOX. 96h	3	68%	81597	86285	0,091
HT. 24h	3	66%	28506	77554	<0,0001**
HT. 48h	3	68%	71181	81391	0,0037**
HT. 96h	3	67%	81597	74601	0,0086**
TAM+DOC. 24h	5	74%	28506	25696	0,21
TAM+DOC. 48h	5	79%	71181	59369	0,0005**
TAM+DOC. 96h	3	69%	81597	82869	0,723
TAM+DOX. 24h	3	73%	28506	28176	0,94
TAM+DOX. 48h	6	69%	71181	75337	0,279
TAM+DOX. 96h	4	75%	81597	81254	0,94
TAM+HT. 24h	4	69%	28506	40470	<0,0001**
TAM+HT. 48h	4	76%	71181	67088	0,085
TAM+HT. 96h	3	75%	81597	64980	<0,0001**
HT+DOC. 24h	3	71%	28506	45480	<0,0001**
HT+DOC. 48h	3	69%	71181	76189	0,12
HT+DOC. 96h	3	71%	81597	78956	0,164
HT+DOX. 24h	3	64%	28506	50022	<0,0001**
HT+DOX. 48h	3	68%	71181	82492	0,003**
HT+DOX. 96h	3	68%	81597	89849	0,001**

MN: Modal Number; p: Student's *t*-test. **p* < 0,05; ** *p* < 0,01



Anexo 7: Figure 1. Representative FISH images of the KPL4 breast cancer cells after A) DOC treatment and B) TAM+DOC treatment. Three-color FISH was performed on nuclei spreads for chromosomes 2, 8 and 11 and, chromosomes 3, 15 and 17 using centromeric probes (CEP) labeled with different spectrum colors: spectrum orange for CEP2 and CEP3; spectrum green for CEP8 and CEP17; and spectrum aqua for CEP11 and CEP15. Interphase nuclei at each treatment time point are indicated. Ctrl: Control, untreated cells.