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CLINICAL TRIAL

Residual risk stratification of Taiwanese breast cancers following curative therapies with the extended concurrent genes signature

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Abstract

Introduction The aim of the study was to perform digital RNA counting to validate a gene expression signature for operable breast cancers initially treated with curative intention, and the risk of recurrence, distant metastasis, and mortality was predicted.

Methods Candidate genes were initially discovered from the coherent genomic and transcriptional alternations from microarrays, and the extended concurrent genes were used to build a risk stratification model from archived formalin-fixed paraffin-embedded (FFPE) tissues with the NanoString nCounter.

Results The extended concurrent genes signature was prognostic in 144 Taiwanese breast cancers (5-year relapse-free survival: 89.8 and 69.4% for low- and high-risk group, log-rank test: $P = 0.004$). Cross-platform comparability was evidenced from significant and positive correlations for most genes as well as equal covariance matrix across 64 patients assayed for both microarray and digital RNA counting.

Discussion Archived FFPE samples could be successfully assayed by the NanoString nCounter. The purposed signature was prognostic stratifying breast cancer patients into groups with distinct survival patterns, and clinical applicability of the residual risk model was proved.

Keywords Breast cancer · Residual risk · Extended concurrent genes · Digital RNA counting · Prognostic signature

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Introduction

Breast cancer is the most common female malignancy in Taiwan and treatment outcomes have improved enormously with early detection and advancements in adjuvant therapy [1, 2]. Unfortunately, a subset of early stage breast cancer still suffers from recurrence, metastasis, or even cancer-specific death despite treatment improvement, and conventional pathological factors fail to provide sufficient explanatory power accounting for the observed prognostic discrepancy.

In the past decade, we had conducted high-throughput microarray experiments at genomic and transcriptional levels to detect copy number variation (CNV) as well as gene expression (GE) profiles. Our projects focused on several aspects of sporadic breast cancers in Taiwan, including concurrent analyses of comparative genomic hybridization (CGH) and GE microarray data, bioinformatics algorithm for microarray classification, and trans-ethnic application of molecular taxonomy [3–6]. Based on the coherent genes across chromosomal and transcriptional alterations, the 32-gene extended concurrent genes signature was purposed as being prognostic among 1145 breast cancer microarray experiments, including discovery cohort of 83 Taiwanese breast cancers for concurrent genes, and 327 Taiwanese and 735 Western sporadic breast cancers for independent leading edge analyses [7].

One drawback of microarray-based multi-gene signature is the prerequisite of fresh cancerous tissue, which may impede wide clinical application. Second, when additional samples are recruited and incorporated into the existing cohort, there remains a risk of re-classification of predicted group as both normalization and classification algorithm are inevitably influenced by the new comer [8, 9]. Third, after determining the identity of constitutional genes, an updated and more efficient gene expression assessment assay with modern technology should be adopted, to replace the hypothesis-driven and obsolete oligonucleotide microarray [10].

The aim of the project was to take advantage of digital RNA counting for gene expression signature synthesis and to develop a predictive model for operable breast cancers initially treated with curative intention, and the risk of recurrence, distant metastasis, as well as mortality was speculated. The validity of transition from microarray to digital RNA counting was ascertained from samples assayed for both platforms as well as independent breast cancer samples.

Materials and methods

Extended concurrent genes signature

The discovery of concurrent genes and synthesis of the extended concurrent genes signature had been published elsewhere and a brief summative description was given here [3, 7]. There were 1584 concurrent genes from 29 Taiwanese breast cancers assayed for both CGH and GE microarrays, and enriched concurrent gene sets for disease-free survival were identified independently from our 83 GE arrays (GSE48391) and one study with Han Chinese origin (GSE20685) and three series of Western countries (GSE7390, GSE2034 and GSE3494) [11–15]. Constitutional genes were selected from leading edge analysis across all enrolled experiments, while supervised partial least square (PLS) regression was used to derived predictive model for relapse-free survival [5, 6]. Prognostic discrepancy was observed between high-risk and low-risk patients predicted with the extended concurrent genes signature [7] (data not shown, manuscript in submission).

Breast cancer samples recruitment

Eligible patients were recruited after consulting cancer registry to identify those diagnosed and operated between 2010 and 2014 with curative intension. The design, purpose was explained to all participants by investigators (CCH and CSH) and written informed consent was pursued before sample collection. As cross-platform comparability was one major interest of current study, breast cancers previously assayed with oligonucleotide microarray with adequate archived surgical pathology were prioritized.

Demographic and clinical features were obtained from cancer registry. Estrogen receptor (ER) and progesterone receptor (PR)-positive status was determined with the presence of at least 10% of nuclei with positive immunohistochemistry (IHC) staining, and breast samples displaying low ER positivity (1–9% of nuclei with positive stains) were not considered as in previous discovery study [3]. Human epidermal growth factor receptor II (HER2) status was determined following the ASCO and CAP guidelines [16]. Those with IHC 3+ and IHC 2+ with fluorescence in situ (FISH) hybridization amplification were categorized into HER2 over-expression. For sample size estimation, we planned to enroll 30 cases per year with a total of 120 and around 30 of them were relapsing/metastatic breast cancers.

Nucleic acid extraction

RNA abundance in formalin-fixed, paraffin-embedded (FFPE) tumor tissue was measured. Archived pathological slides or tissue blocks were retrieved from department of pathology. FFPE sections and accompanied hematoxylin and eosin (H&E) stained slides were reviewed by one certified pathologist (CYL) to select areas that contained at least 70% tumor cells for RNA extraction. When only pathological slide was available, the target tissue must cover 30% of the surface area and the excess paraffin was

removed using a scalpel prior to extraction. Paraffin was removed from specimens by xylene extraction then by ethanol washes.

Total RNA was isolated and purified from each 10-µm-thick roll using the RNeasy FFPE Kit (Qiagen, Hilden, Germany) as per manufacturer's instructions with modifications. Standard de-crosslinking and column purification steps were performed to remove proteins and other cellular components prior to RNA elution in water. The quantity and quality of extracted RNA were determined by the NanoDrop spectrophotometers (Thermo Fisher Scientific, MA, USA) using a wavelength spectrum of 220–320 nm, evaluating the 260/280 ratio, and by separation on an Agilent BioAnalyzer (Agilent, Santa Clara, CA). RNA input was scaled based on DV 300 value to 150 ng (150 ng/DV300 × 100) and proceeded according to the nCounter MAX assay user manual. For quality control, a minimum DV 300 of 20 was required.

Digital RNA counting

Gene expression levels were measured in total RNA isolated from FFPE tissues using the NanoString nCounter (NanoString Technologies Inc., Seattle, WA). The underlying chemistry included target-specific reporter and capture probes, and the collective CodeSet hybridized to regions of interest with covalently attached and target-specific sequences.

Normalization of raw transcript counts was performed by dividing the geometric mean of six housekeeper-control genes: *ACTB, G6PD, RPLP0, TBP, TFRC* and *UBB*. Following positive control normalization, housekeeper-normalized transcript counts were log2 transformed and data were row z-score standardized before further analysis. Prediction analysis of microarray 50 (PAM50) analysis was performed using the published quantitative real-time PCR (qRT-PCR) centroids on the normalized log2 gene expression data before z-standardization [17]. nSolver version 4.0 (NanoString Technologies Inc., Seattle, WA) was used for data import, quality control, and outputted normalized expression values. Normalization steps included (1) reducing technical variation by adjusting each sample's counts based on its relative value to the geometric mean of all samples; (2) correcting background count levels by subtracting from each sample's count the mean plus two standard deviations of counts from negatives controls; and (3) normalizing for sample RNA content using the geometric mean of expression levels from the six reference genes [18, 19]. Downstream analyses were carried out by BRB Array-Tools version 4.6.1 (National Cancer Institute, Bethesda, MD) for visualization purpose and SAS (SAS Institute Inc., Cary, NC) version 9.4 for risk group classification and survival analyses [20].

Cross-platform comparability and measurement invariance

Breast cancer samples assayed for both the Affymetrix GeneChip Human Genome U133 Plus 2.0 microarray (Thermo Fisher Scientific, MA, USA) and the customized NanoString nCounter BCeC Sig CodeSet provided the source for testing cross-platform comparability and measurement invariance. Concordance of gene expression measured between oligonucleotide microarray, which was adopted during the development of the purposed signature, and digital RNA counting, which deployed the CodeSet, was evaluated with the Spearman's correlation coefficient statistics. Gene expression values measured by digital RNA counting were also predicted by corresponding genes measured by microarray through a linear regression model with a prior z-transformation within each platform.

Multi-group structural equational modeling (SEM) was conducted to appraise the equality of two covariance matrices for measurement invariance [21, 22]. The covariance matrices of two independent groups were tested for equality as they were constrained to be the same under the null hypothesis. The null hypothesis was:

$$H_0 : \sum_1 = \sum_2,$$

where $\sum_1$ and $\sum_2$ were the population covariance matrices of the two independent groups (gene expression profiles measured independently by microarray and digital RNA counting in current study). Lagrange multiplier (LM) statistics were calculated for releasing variances/covariances constraints. LM statistics estimated the reduction of model fit Chi-square statistic if the constraint put on the corresponding parameter was released; only P-value < 0.001 was considered for multiple testing correction.

Prognostic signature

Gene expression classification with PLS regression had been described elsewhere, which was essentially the linear transformation of high-dimensional gene expression data into quite a few orthogonal latent factors [5]. PLS was used for dimension reduction and the first latent X-factor (also known as gene component) was constructed for the constitutional genes. PLS regression maximized the covariance between the predictors (signature genes) and the responder (dichotomous high-/low-risk group). Missing values in gene expression were imputed with expectation–maximization algorithm [23]. Leave-one-out cross validation was performed to prevent model over-fitting. The threshold for high-/low-risk group was determined

from the 75th percentile of the first extracted latent factor (*X*-score) considering the average relapse rate among all breast cancer subtypes. The predictors and the response variable were centered and scaled to have mean zero and standard deviation one.

Results

Targeted gene expression

A custom-designed NanoString nCounter gene expression CodeSet, BCeC Sig, was established, for which transcriptional profiles of targeted genes were evaluated with digital RNA counting. Signature genes included 32 genes of the extended concurrent genes signature (Supplementary Table 1 for details of CodeSet design regarding interrogated genes). All samples with expression data passing quality control were included for downstream analyses. To classify intrinsic molecular subtype, we applied a centroid-based clustering algorithm based on the single sample prediction (SSP) classification developed by Parker et al. known as PAM50 [17].

Assayed Taiwanese breast cancers

RNA was extracted successfully from FFPE samples with low failure rates of nucleic acids degradation, and digital RNA counting experiments were performed with the customized BCeC Sig CodeSet running on the NanoString nCounter. A total of 144 Taiwanese breast cancers were successfully assayed. Table 1 details clinical characteristics. To further evaluate the cross-platform comparability, we tried to enroll breast cancers ($n=64$) who had also been assayed for the Affymetrix microarrays. Supplementary Table 2 shows individual clinical features of each enrolled subject.

Cross-platform comparability

To understand the comparability in mRNA abundance between the measurement conducted by Affymetrix oligonucleotide microarray and NanoString nCounter-based digital RNA counting, 64 breast samples assayed for both platforms were retrieved and compared. Concordance between both platforms was evaluated within the same subject, and Table 2 showed Spearman's correlation coefficients of each element constituting the extended concurrent genes signature. Most genes showed a significant and positive correlation, except

Table 1 Clinical characteristics of 144 Taiwanese breast cancers

	IHC subtype					*P*-value
	HR+/HER2+	HR+/HER2−	HR−/HER2+	HR−/HER2−	Missing	
Relapse-free survival event						
Disease-free	22	66	21	9	4	0.77
Relapse	4	11	3	2	2	
Total	26	77	24	11	6	
All-cause mortality event						
Death	20	65	19	4	6	< 0.01
Alive	6	12	5	7	0	
Total	26	77	24	11	6	
PAM50 SSP						
Basal	4	7	8	7	2	< .0001
HER2	13	4	12	4	1	
Lum-A	5	38	3	0	2	
Lum-B	4	28	1	0	1	
Total	26	77	24	11	6	
Follow-up time (year)						
N	26	77	24	11	6	0.49
Mean	3.5	3.7	3.4	2.6	3.2	
SD	2.5	2.3	2.2	1.8	4.2	
Minimum	0.3	0.3	0.7	0.2	0.8	
Maximum	9.4	7.4	6.8	5.4	11.6	

IHC immunohistochemistry, *HR* hormone receptor, *HER2* human epidermal growth factor receptor II, *PAM50 SSP* Prediction Analysis of Microarray 50 single sample predictor

Table 2 Spearman's correlation coefficients between mRNA abundance measured by oligonucleotide microarray and digital RNA counting

Symbol	Spearman's correlation coefficient	*P*-value
ASPM	0.72	< .0001
ATP9A	0.67	< .0001
AURKA	0.66	< .0001
CDC20	0.76	< .0001
CENPF	0.71	< .0001
CSE1L	0.56	< .0001
DENND2D	0.56	< .0001
EDN2	0.70	< .0001
EIF4EBP1	0.72	< .0001
FBXO5	0.54	< .0001
GINS1	0.80	< .0001
GRB7	0.64	< .0001
HLA-DOB	0.52	< .0001
IDH3A	0.26	0.04
IKZF1	0.56	< .0001
KIF14	0.75	< .0001
KIF2C	0.74	< .0001
LACTB2	0.57	< .0001
MRPS2	0.13	0.3231
OSBPL2	0.06	0.6535
PNRC1	− 0.11	0.3794
PPARD	− 0.03	0.8429
PPFIA1	0.07	0.5614
PTK6	0.54	< .0001
RECQL4	0.60	< .0001
S100PBP	0.24	0.0518
SERPINB3	0.13	0.4115
SLC16A3	0.37	0.0025
SLC25A1	0.60	< .0001
STARD3	0.65	< .0001
UBE2C	0.56	< .0001
UBE2V2	0.53	< .0001

Table 3 Transcriptional abundance measured by digital RNA counting regressed on gene expression values measured by oligonucleotide microarray

Symbol	Intercept	Regression coefficient	*P*-value
ASPM	− 1.21E–15	0.69	3.31E–10
ATP9A	− 2.74E–16	0.74	2.56E–12
AURKA	6.79E–17	0.62	5.14E–08
CDC20	3.88E–16	0.75	7.28E–13
CENPF	7.67E–16	0.71	6.29E–11
CSE1L	− 7.42E–15	0.53	8.42E–06
DENND2D	− 2.85E–15	0.51	1.9079E–05
EDN2	− 9.27E–16	0.67	9.71E–10
EIF4EBP1	− 1.46E–15	0.79	1.06E–14
FBXO5	3.06E–16	0.53	7.36E–06
GINS1	6.45E–16	0.75	1.02E–12
GRB7	− 1.02E–16	0.77	1.42E–13
HLA-DOB	8.24E–16	0.50	2.2438E–05
IDH3A	1.44E–15	0.38	0.00199207
IKZF1	7.24E–16	0.60	1.42E–07
KIF14	1.07E–15	0.72	2.31E–11
KIF2C	− 1.87E–15	0.74	2.31E–12
LACTB2	1.32E–15	0.60	1.32E–07
MRPS2	1.06E–15	0.11	0.39764837
OSBPL2	4.04E–15	0.02	0.90384917
PNRC1	1.89E–15	− 0.12	0.33731995
PPARD	− 2.96E–15	− 0.14	0.25738522
PPFIA1	3.23E–15	0.05	0.68818773
PTK6	2.20E–15	0.61	1.10E–07
RECQL4	− 1.39E–15	0.58	6.70E–07
S100PBP	− 6.89E–16	0.34	0.00644616
SERPINB3	3.69E–16	0.13	0.29052746
SLC16A3	5.27E–15	0.47	9.6126E–05
SLC25A1	3.08E–15	0.63	3.04E–08
STARD3	− 1.26E–15	0.85	3.58E–19
UBE2C	3.24E–15	0.58	5.05E–07
UBE2V2	1.50E–17	0.59	2.79E–07

for *MRPS2*, *OSBPL2*, *PNRC1*, *PPARD*, *PPFIA1*, *S100PBP,* and *SERPINB3*.

If mRNA measured by nCounter was treated as the response variable (Y) and was predicted by a linear function of the regressors (X_s), which were mRNA transcriptions measured by microarray, the intercept and slope (coefficient) estimated are detailed in Table 3 with each signature gene centered and scaled to have mean zero and standard deviation one within each platform. Except for *PNRC1* and *PPARD* (negative slopes) and *MRPS2*, *OSBPL2*, *PPFIA1* and *SERPINB3* (insignificant regression coefficients), most investigated genes showed a concordant trend. Normalized (not standardized) gene expression data of both gene expression assays were provided in Supplementary Tables 2 and 3.

Measurement invariance

To further evaluate the measurement models of distinct gene expression assays as well as the trans-platform comparability, we took advantage of multi-group SEM to test equality of two covariance matrices using a multiple-group analysis. Initially we tested if these two groups had the same covariance matrix, and the covariance matrix itself was unconstrained as the default setting of all non-redundant elements in the covariance matrix being free parameters. The model fit indices showed a Chi-square value of 747.0458 ($df = 528$) and a P-value < 0.0001 [Root mean square error of approximation (RMSEA): 0.0811], which rejected the null hypothesis of equality of the covariance matrices.

LM statistics were consulted to release variances/covariances constraints. Covariance between *UBE2C* and *KIF2C* ($P=0.0003$, LM statistic: 13.00361) and variance of *RECQL4* ($P=0.0007$, LM statistic: 11.36518) and *UBE2V2* ($P=0.0058$, LM statistic: 7.61829). Serial modifications and model fit indices showed much improvement toward equal covariance (data not shown).

We also trained the model from 144 breast cancers undergoing digital RNA counting. Supplementary Fig. 1 showed the conceptual structure of extended concurrent genes signature.

Survival analysis

Predictor (signature gene) weights and loadings are detailed in Supplementary Table 4. The threshold was set to the 75th percentile of the first *X*-score (1.9459). During the up to 11.6 years of follow-up (median follow-up time: 5.5 year for low-risk group and 4.6 for high-risk group), there were 11 events (local recurrence, distant metastasis, or breast cancer-specific death) in each group, resulting in relapse-free survival rate of 89.8 and 69.4% for low- and high-risk group respectively (log-rank test: $P=0.004$). For all-cause mortality, there were 17 and 13 fatal events for the low- and high-risk group, and the overall survival rate was 91 and 23% respectively (log-rank test: $P=0.0294$). Figures 1 and 2 show relapse-free and overall survival of Taiwanese breast cancers stratified by the extended concurrent genes signature assayed by digital RNA counting (Supplementary Figs. 2 and 3 show the same plots stratified by the PAM50 subtypes). Clustering heatmaps are displayed in Fig. 3 and Supplementary Fig. 4 (samples ordered by relapse-free status). There was no interaction between predicted risk group and IHC subtype (Table 1). After multi-variate analysis adjusted for clinical hormone receptor and HER2 status, the predicted risk group remained significant with hazard ratios of 4.1 and 2.4 reported for relapse-free survival and overall survival ($P<0.01$ and $P=0.02$, respectively).

Discussion

In current study, the prognostic performance of the extended concurrent genes signature, initially developed from oligonucleotide microarrays, was ascertained from the NanoString nCounter-based digital RNA counting gene expression panel. The cross-platform comparability was evidenced from 64 Taiwanese breast cancers assayed for both platforms, and prognostic relevance was observed among 144 patients showing distinct survival patterns stratified by the predicted risk groups.

Nowadays adjuvant therapies following curative surgery for early breast cancer are determined from predictive (some are also prognostic) factors such as IHC assays for ER, PR, HER2, Ki-67 as well as morphology of nuclear grade [24]. Despite enormous advancement in hormone manipulation, cytotoxic chemo-, and targeted therapy, there remains a substantial proportion of early breast cancers who still suffer from local recurrence, distant metastasis, or breast cancer associated death following curative surgery [25]. On the

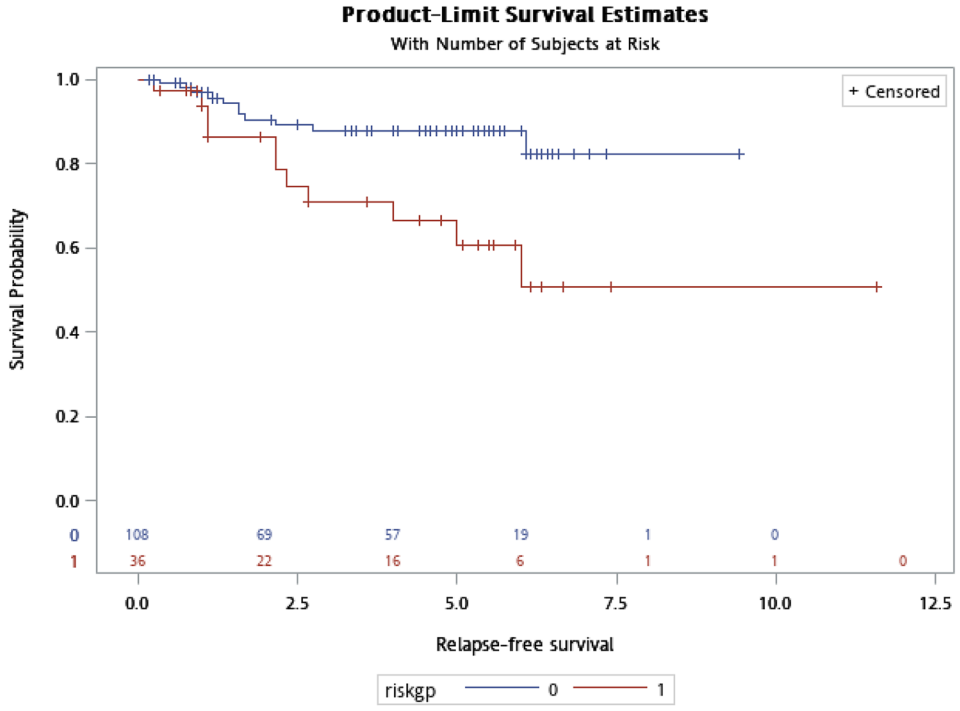

Fig. 1 Relapse-free survival between the high- (riskgp = 1) and low-risk (riskgp = 0) group defined by the extended concurrent genes signature (log-rank test: 0.004, riskgp: risk group)

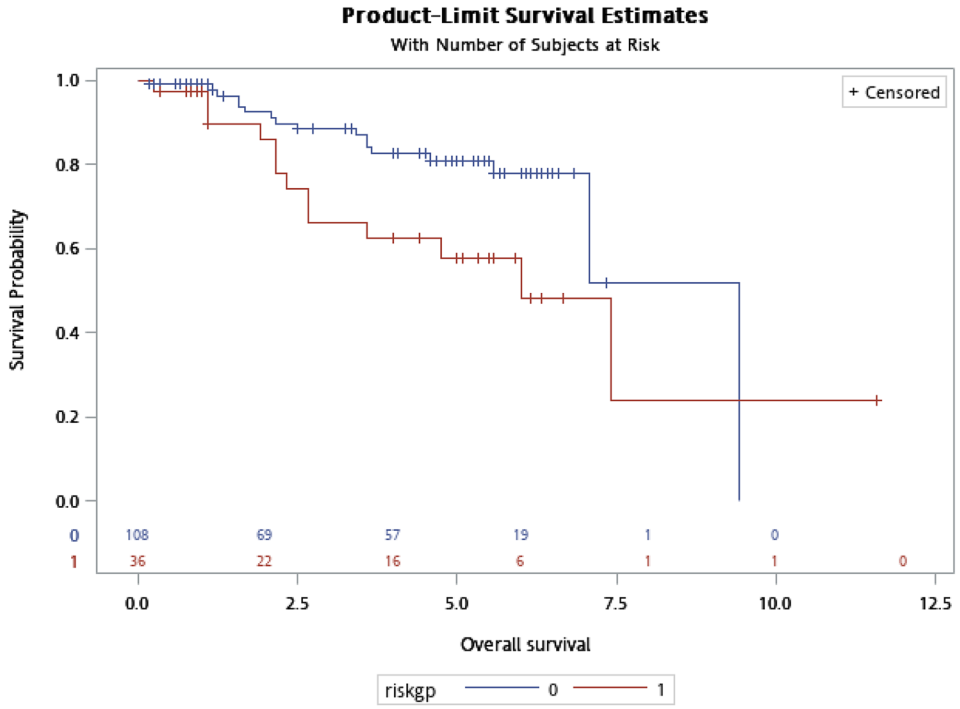

Fig. 2 Overall survival between the high- (riskgp = 1) and low-risk (riskgp = 0) group defined by the extended concurrent genes signature (log-rank test: 0.0294, riskgp: risk group)

other hand, these conventional pathology-based factors fail to provide complete explanation for the observed prognostic discrepancy within each subtype such as one-fourth of HER2 over-expressed breast cancers eventually develop resistance to trastuzumab, a humanized monoclonal anti-HER2 antibody [26]. Therefore, accurate risk assessment becomes a must for effective surveillance for breast cancer survivors and risk management.

In the past decade, microarray analyses have redefined breast cancer as a union of distinct molecular subtypes and a couple of molecular taxonomies have been established, with most displaying prognostic and some with predictive power from retrospective cohort or randomized controlled trials [17, 27–31]. Most microarray-based or RT-PCR-based multi-gene expression biomarkers published so far are restricted to ER-positive and limited nodal involvement (N0/1) subpopulation, which may limit clinical applicability [32, 33].

Our published concurrent genes and the updated extended concurrent genes signatures are molecular biomarkers which capture the genetic aberrations inherited in breast cancer pathogenesis. Concurrent genes are those with coherent patterns between genomic and transcriptional alternations through integrating analyses [3, 7]. The discovery cohort included 31 CGH and 83 GE microarrays, of which 29 breast cancers were assayed for both platforms. In addition, targets were also determined by Genomic Identification of Significant Targets in Cancer (GISTIC) from CGH microarrays [34]. A total of 1584 concurrent genes and genes with significant GISTIC scores were used to derive signatures, which were enriched concurrent gene sets across 83 GE arrays and one series with Han Chinese origin as well as three studies of Western origin [11–15]. Consensus from leading edge analysis was followed by supervised PLS regression predictive model for relapse-free survival and prognostic discrepancy was observed between predicted high-risk and low-risk group patients [7].

Regarding the published multi-gene signature for breast cancer prognosis, there are microarray-based such as the 70-gene MammaPrint (Agendia, Morgan Irvine, CA), RT-PCR-based 21-gene Oncotype DX (Genomic Health, Redwood City, CA) and 12-gene EndoPredict (Myriad Genetics, Salt Lake City, UT), and NanoString nCounter-based 50-gene Prosigna (Veracyte, South San Francisco, CA) [27–31, 35]. While most multi-gene signatures have used microarrays for candidate gene discovery during development, the final version usually adopt more efficient RT-PCR or digital RNA counting for gene expression measure. Therefore, cross-platform comparability should be critically evaluated to enhance prognostic validity.

Digital RNA counting was determined for the deployment of the extended concurrent genes signature as easily degraded nucleic acid from archived FFPE samples could be handled by the NanoString nCounter [18, 19]. Although nowadays it is not novel to perform gene expression profiling from FFPE samples with the nCounter system, it was expected that nucleic acid degradation became a major issue in current study as RNA was extracted from breast cancers operated between 2010 and 2014. Consequently, it is necessary to select a subset of study samples whose fresh frozen

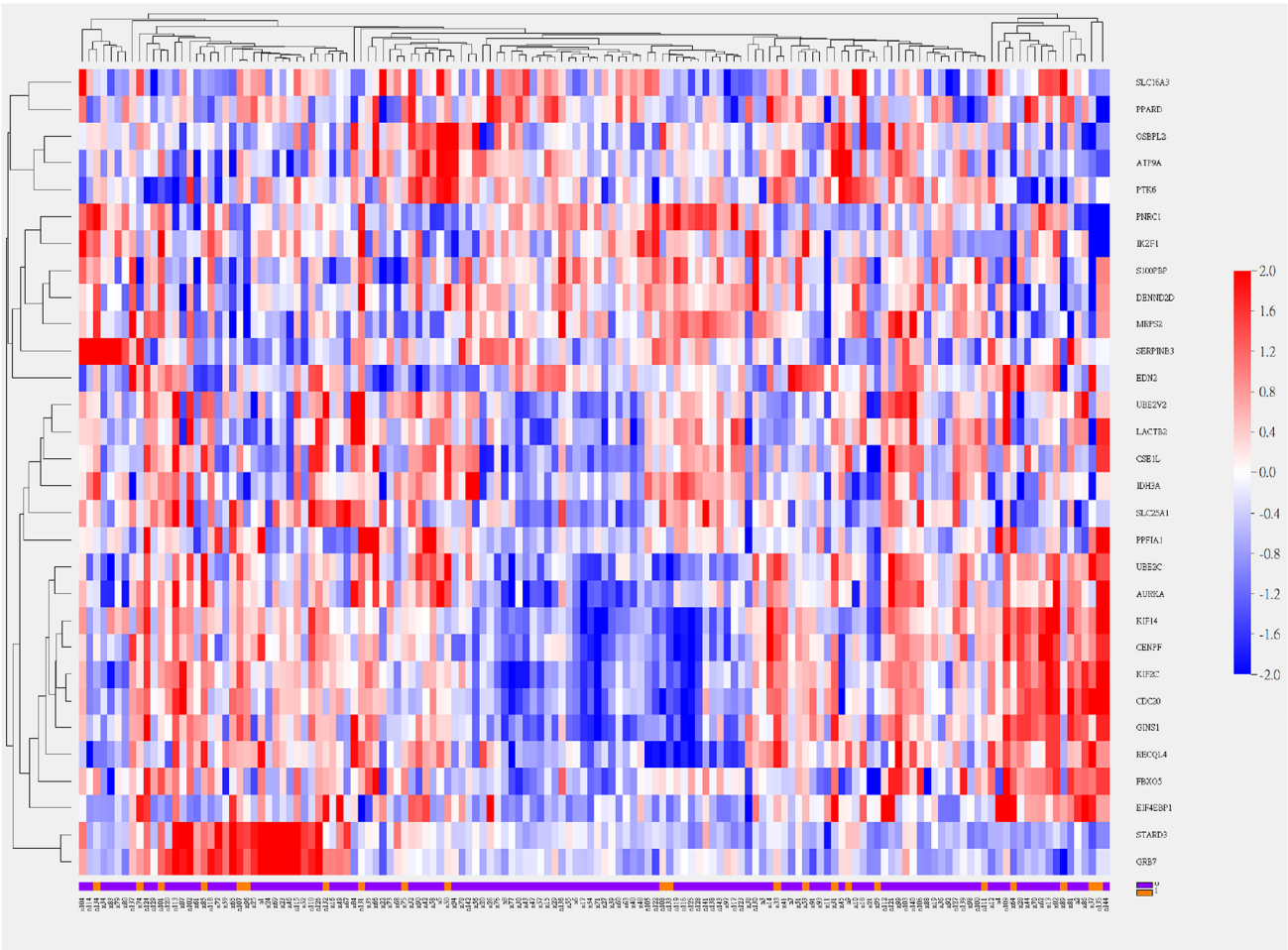

Fig. 3 Two-way hierarchical clustering heatmap of the extended concurrent genes and breast cancers. Average linkage and one-correlation matric were used for distance calculation. *X*-axis list breast cancer sample names and *purple block* indicated disease-free and *brown block* indicated relapse cases

tissues had been assayed for gene expression with microarray and to check whether the expression patterns of signature genes were similar between microarray and nCounter. Molecular barcodes provide a digital detection capable of highly multiplexed analyses. Fresh frozen tissues are needed for most microarray hybridization, while snapped-frozen samples are limited in real-world scenarios. Archived pathological specimens provide a precious source to validate the residual risk prediction model for breast cancers managed with multi-modalities including surgery, chemo-, endocrine, and targeted therapy.

Initially mRNA measurement concordance between oligonucleotide microarray and digital RNA counting was evaluated from 64 breast cancers assayed for both platforms and most (25 out of 32) signature genes showed a positive and significant correlation coefficient. When nCounter measured mRNA abundance was predicted from the one measured by microarray, most (26 out of 32) signature genes also showed a significant and positive regression coefficient. Since gene expression values were standardized within each platform before regression, all intercept estimates were nearly zero.

In addition to numeric correlation of each constitutional gene, we also took advantage of multi-group SEM to test equality of two covariance matrices. When a theoretical model, in our case the extended concurrent genes signature, was justified as a good approximation to the transcriptional profiles measured with a specific instrument such as the oligonucleotide microarray, the next step was to ask whether the same construct held true across heterogeneous groups differed in assessing methods. It deserved notice that mean structure analysis was not deciphered as data were standardized (z-transformed) within each gene expression assay independently.

Although the significant P-value ($P < 0.0001$) rejected the null hypothesis of common covariance matrix among the two independent assays, the Chi-square test itself might not be meaningful here. This value was calculated from Chi-square value and model degrees of freedom,

and the null hypothesis was that the predicted model fitted the observed data well. However, Chi-square test is very sensitive to sample size and the larger the sample size, the greater the chance to get a significant Chi-square test. Some even recommend using the Chi-square divided by the degrees of freedom χ^2/df less than 5 as a practical alternative index [36]. Our modest sample size of 144 might render the Chi-square test for model fitness unreliable. On the other hand, 0.0811 of RMSEA indicated a slight deviation from good model fit, while LM statistics suggested releasing equal covariance constraint on covariance of *UBE2C* and *KIF2C* as well as equal variance constraints on *RECQL4* and *UBE2V2* between microarray and digital RNA counting platform (all P-values < 0.01). Serial modifications with more general models might result in enhanced comparability and much equal covariance matrices of these two gene expression assays. At the same time, distinct regression coefficients for each signature gene were also required for the novel digital RNA counting assay, as well as recalibrated threshold for the defined high-/low-risk group.

The extended concurrent genes signature, currently transformed and conducted on a NanoString nCounter gene expression panel, could facilitate our understanding of breast cancer residual risk after curative surgery as prognostic discrepancy was observed between the high- and low-risk groups. Optimized performance on FFPE samples further guaranteed wide clinical applicability. Digital detection of individual targets was achieved through unique molecular barcodes and reported probe counts representing precise and accurate gene expression measurements. The customized BCeC Sig CodeSet was trained from 144 Taiwanese breast cancers and prospective analysis of this retrospective cohort showed prognostic power independent of clinical subtypes such as ER and HER2 status. More efficient breast cancer prognostic prediction through digital RNA counting of signature genes selected form microarray experiments is evidenced. As these patients were treated following the contemporary guidelines with curative intention, the incremental risk assessed may contribute substantially to the understanding of survival discrepancy observed within each clinical stratum.

There were some limitations of the study. First, both gene expression assays were not performed simultaneously. Oligonucleotide microarrays were conducted in a prospective manner for fresh frozen samples, while nCounter assays were performed for retrospectively retrieved pathological archives. Second, the sample size of 144 breast cancers was only modest and prospective study to evaluate the prognostic power of the purposed signature is warranted in the future.

In conclusion, the proposed breast cancer residual risk model, composed of the extended concurrent genes signature, was believed to provide clinical applicability and substantial benefits for Taiwanese breast cancer patients in terms of personalized medicine.

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Compliance with ethical standards

Conflict of interest All authors declare that there is no conflict of interest. The whole study protocol was reviewed and approved by the institutional review board of Cathay General Hospital.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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2022 Journal Performance Data for: BREAST CANCER RESEARCH AND TREATMENT

ISSN
0167-6806

EISSN
1573-7217

JCR ABBREVIATION
BREAST CANCER RES TR

ISO ABBREVIATION
Breast Cancer Res. Treat.

Journal Information

EDITION
Science Citation Index Expanded (SCIE)

CATEGORY
ONCOLOGY - SCIE

LANGUAGES
English

REGION
USA

1ST ELECTRONIC JCR YEAR
1997

Publisher Information

PUBLISHER
SPRINGER

ADDRESS
ONE NEW YORK PLAZA, SUITE 4600 , NEW YORK, NY 10004, UNITED STATES

PUBLICATION FREQUENCY
18 issues/year

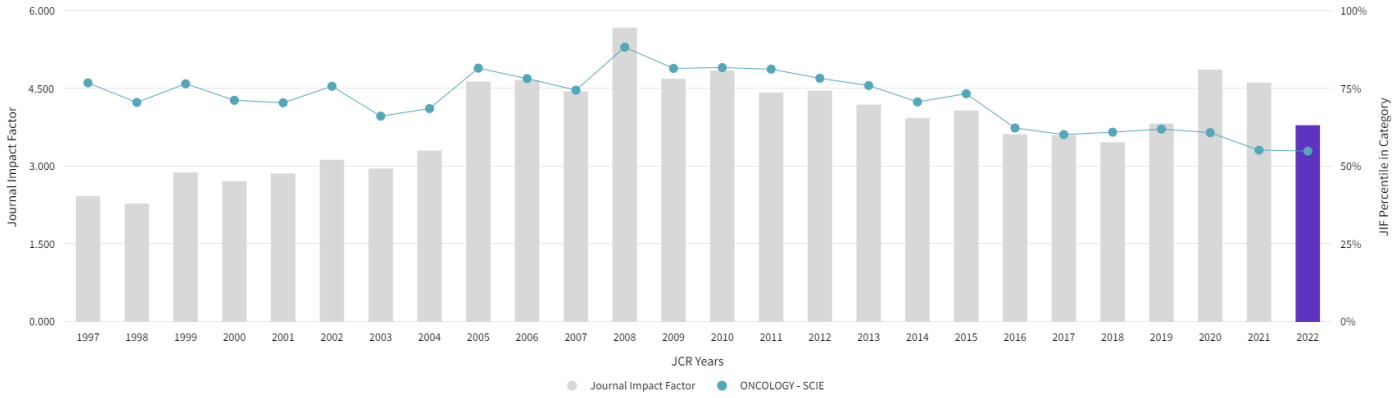
Journal's Performance

Journal Impact Factor

The Journal Impact Factor (JIF) is a journal-level metric calculated from data indexed in the Web of Science Core Collection. It should be used with careful attention to the many factors that influence citation rates, such as the volume of publication and citations characteristics of the subject area and type of journal. The Journal Impact Factor can complement expert opinion and informed peer review. In the case of academic evaluation for tenure, it is inappropriate to use a journal-level metric as a proxy measure for individual researchers, institutions, or articles. Learn more

2022 JOURNAL IMPACT FACTOR	2022 JOURNAL IMPACT FACTOR WITHOUT SELF CITATIONS
3.8	3.7

Journal Impact Factor Trend 2022



Journal Impact Factor is calculated using the following metrics

Citations in 2022 to items published in 2020 (**2,448**) - 2021 (**1,112**)		**3,560**		
Number of citable items in 2020 (**530**) + 2021 (**398**)	=	**928**	=	**3.8**

Journal Impact Factor without self cites is calculated using the following metrics

Citations in 2022 to items published in 2020 (**2,448**) + 2021 (**1,112**) - Self Citations in 2022 to items published in 2020 (**85**) + 2021 (**52**)		**3,560 - 137**		
Number of citable items in 2020 (**530**) + 2021 (**398**)	=	**928**	=	**3.7**

Journal Impact Factor Contributing Items

Citable Items (928)

TITLE	CITATION COUNT
Genetic polymorphisms in DNA repair genes XRCC1 and 3 are associated with increased risk of breast cancer in Bangladeshi population Authors: Howlader, Nupur Rani;Rahman, Md. Mostafizur;Hossain, Md. Amir;Sultana, Razia;Hossain, Syed Mozammel;Mazid, Md. Abdul;Rahman, Md. Mustafizur Volume: 182 Accession number: WOS:000541329200003 Document Type: Article	160
Patient-reported treatment delays in breast cancer care during the COVID-19 pandemic Authors: Papautsky, Elizabeth Lerner;Hamlish, Tamara Volume: 184 Accession number: WOS:000557740800003 Document Type: Article	49
Trends of female and male breast cancer incidence at the global, regional, and national levels, 1990-2017 Authors: Chen, Zhilin;Xu, Lu;Shi, Wenjie;Zeng, Fanyu;Zhuo, Rui;Hao, Xinbao;Fan, Pingming Volume: 180 Accession number: WOS:000518129400002 Document Type: Article	26
Overall survival results from the randomized phase 2 study of palbociclib in combination with letrozole versus letrozole alone for first-line treatment of ER+/HER2-advanced breast cancer (PALOMA-1, TRIO-18) Authors: Finn, Richard S.;Bananis, Eustratios;McRoy, Lynn;Wilner, Keith;Huang, Xin;Kim, Sindy;Slamon, Dennis J.;Ettl, Johannes;Boer, Katalin;Bondarenko, Igor; et al. Volume: 183 Accession number: WOS:000549817500002 Document Type: Article	25
Symptom cluster of pain, fatigue, and psychological distress in breast cancer survivors: prevalence and characteristics Authors: Bjerkeset, Ellen;Rohrl, Kari;Schou-Bredal, Inger Volume: 180 Accession number: WOS:000515001400006 Document Type: Article	22

Showing 1-5 rows of 928 total (use export in the relevant section to download the full table)

Journal Impact Factor Contributing Items

Citing Sources (981)

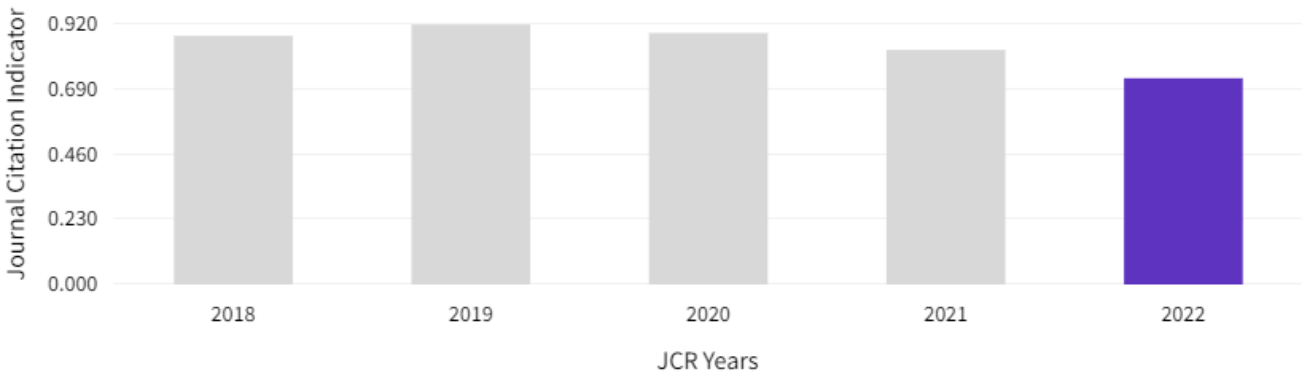
SOURCE NAME	COUNT
CANCERS	174
FRONTIERS IN ONCOLOGY	171
BREAST CANCER RESEARCH AND TREATMENT	137
ANNALS OF SURGICAL ONCOLOGY	60
INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES	58
BREAST	49
SUPPORTIVE CARE IN CANCER	47
BMC CANCER	43
SCIENTIFIC REPORTS	42
CANCER MEDICINE	34
CURRENT ONCOLOGY	30
CLINICAL BREAST CANCER	28
CRITICAL REVIEWS IN ONCOLOGY HEMATOLOGY	26
FRONTIERS IN IMMUNOLOGY	26
FRONTIERS IN PHARMACOLOGY	26
CELLS	25
FRONTIERS IN ENDOCRINOLOGY	24
JOURNAL OF CLINICAL MEDICINE	23
NPJ BREAST CANCER	23
JOURNAL OF CANCER SURVIVORSHIP	22

Showing 1-20 rows of 981 total (use export in the relevant section to download the full table)

Journal Citation Indicator (JCI)

0.73

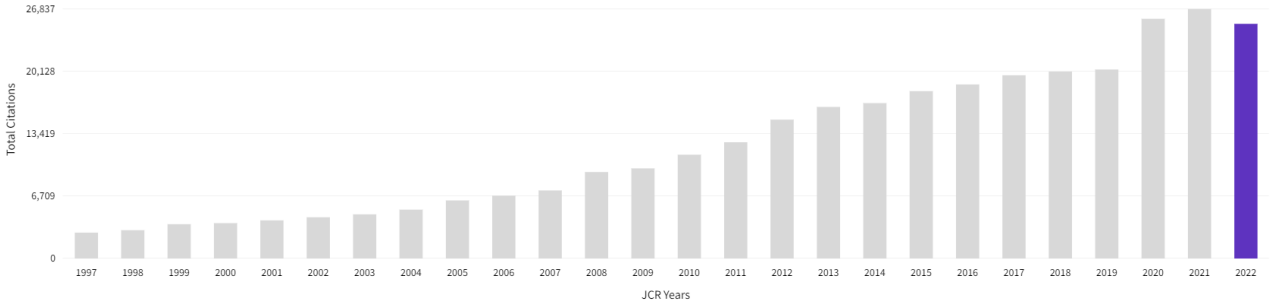
The Journal Citation Indicator (JCI) is the average Category Normalized Citation Impact (CNCI) of citable items (articles & reviews) published by a journal over a recent three year period. The average JCI in a category is 1. Journals with a JCI of 1.5 have 50% more citation impact than the average in that category. It may be used alongside other metrics to help you evaluate journals. Learn more



Total Citations

25,242

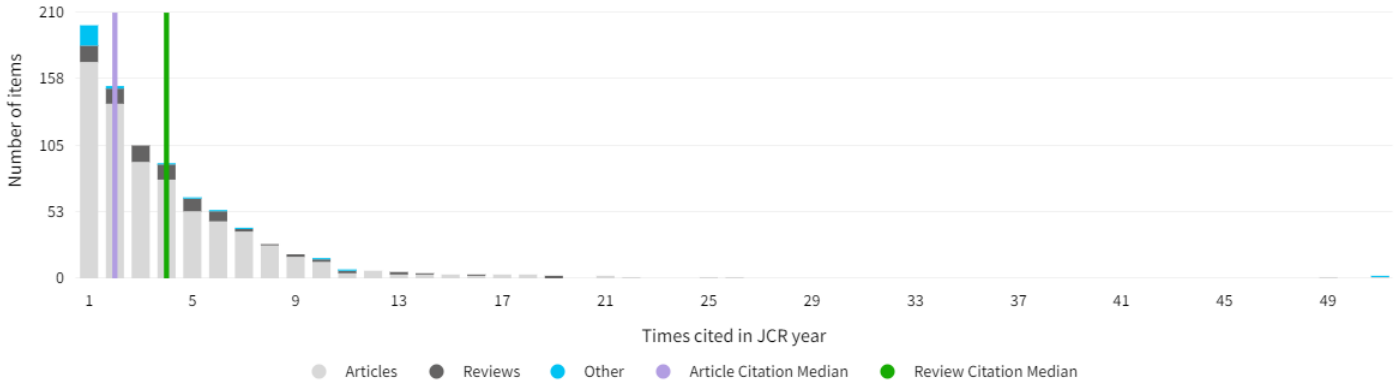
The total number of times that a journal has been cited by all journals included in the database in the JCR year. Citations to journals listed in JCR are compiled annually from the JCR years combined database, regardless of which JCR edition lists the journal.



Citation Distribution

The Citation Distribution shows the frequency with which items published in the year or two years prior were cited in the JCR data year (i.e., the component of the calculation of the JIF). The graph has similar functionality as the JIF Trend graph, including hover-over data descriptions for each data point, and an interactive legend where each data element's legend can be used as a toggle. You can view Articles, Reviews, or Non-Citable (other) items to the JIF numerator. Learn more

ARTICLE CITATION MEDIAN	REVIEW CITATION MEDIAN	UNLINKED CITATIONS
2	4	13



0 times cited

ARTICLES	REVIEWS	OTHER
135	6	227

Open Access (OA)

The data included in this tile summarizes the items published in the journal in the JCR data year and in the previous two years. This three-year set of published items is used to provide descriptive analysis of the content and community of the journal.Learn more

Items

TOTAL CITABLE
1,288

% OF CITABLE OA
26.55%

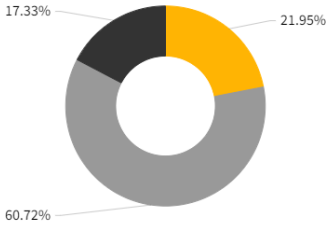
CITABLE

GOLD OPEN ACCESS
342 / 21.95%

SUBSCRIPTION OR BRONZE
946 / 60.72%

NON-CITABLE

OTHER (NON-CITABLE ITEMS)
270 / 17.33%



Citations*

TOTAL CITABLE
3,623

% OF CITABLE OA
26.08%

CITABLE

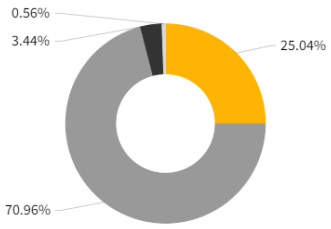
GOLD OPEN ACCESS
945 / 25.04%

SUBSCRIPTION OR BRONZE
2,678 / 70.96%

NON-CITABLE

OTHER (NON-CITABLE ITEMS)
130 / 3.44%

UNLINKED CITATIONS
21 / 0.56%



* Citations in 2022 to items published in (2020-2022)

Rank by Journal Impact factor

Journals within a category are sorted in descending order by Journal Impact Factor (JIF) resulting in the Category Ranking below. A separate rank is shown for each category in which the journal is listed in JCR. Data for the most recent year is presented at the top of the list, with other years shown in reverse chronological order. Learn more

EDITION

Science Citation Index Expanded (SCIE)

CATEGORY

ONCOLOGY

109/241

JCR YEAR	JIF RANK	QUARTILE	JIF PERCENTILE
2022	109/241	Q2	55.0
2021	110/245	Q2	55.31
2020	95/242	Q2	60.95
2019	93/244	Q2	62.09
2018	90/230	Q2	61.09
2017	89/223	Q2	60.31
2016	82/217	Q2	62.44
2015	57/213	Q2	73.47
2014	62/211	Q2	70.85
2013	49/203	Q1	76.11
2012	43/197	Q1	78.43
2011	37/196	Q1	81.38
2010	34/185	Q1	81.89
2009	31/166	Q1	81.63
2008	17/143	Q1	88.46
2007	34/132	Q2	74.62
2006	28/127	Q1	78.35
2005	23/123	Q1	81.71
2004	39/123	Q2	68.70
2003	41/120	Q2	66.25
2002	28/114	Q1	75.88
2001	32/107	Q2	70.56
2000	30/103	Q2	71.36
1999	25/105	Q1	76.67
1998	31/104	Q2	70.67
1997	24/102	Q1	76.96

Rank by Journal Citation Indicator (JCI)

Journals within a category are sorted in descending order by Journal Citation Indicator (JCI) resulting in the Category Ranking below. A separate rank is shown for each category in which the journal is listed in JCR. Data for the most recent year is presented at the top of the list, with other years shown in reverse chronological order.Learn more

CATEGORY

ONCOLOGY

136/317

JCR YEAR	JCI RANK	QUARTILE	JCI PERCENTILE
2022	136/317	Q2	57.26
2021	111/318	Q2	65.25
2020	107/310	Q2	65.65
2019	101/308	Q2	67.37
2018	106/302	Q2	65.07
2017	92/290	Q2	68.45

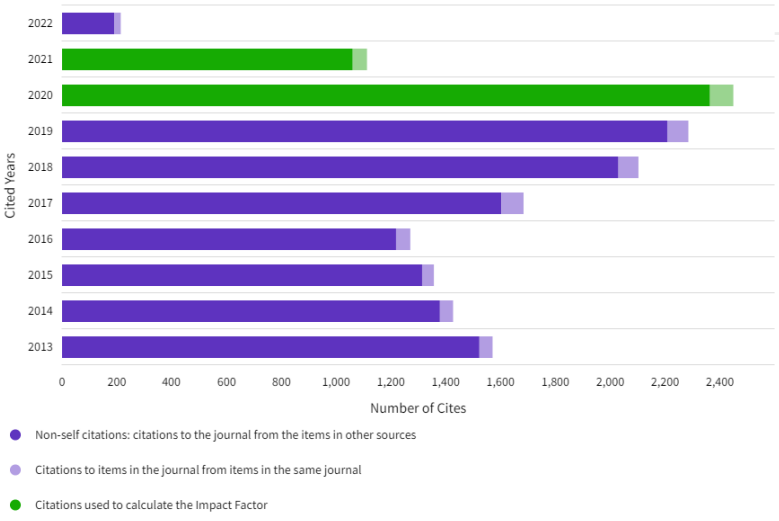
Citation network

Cited Half-life

8.1 years

The Cited Half-Life is the median age of the items in this journal that were cited in the JCR year. Half of a journal's cited items were published more recently than the cited half-life.

TOTAL NUMBER OF CITES	NON-SELF CITATIONS	SELF CITATIONS
25,242	24,414	828



# OF CITES FROM 2022	CUMULATIVE %	# OF CITING SOURCES
25,242 citations	**100.00%**	**2,775 sources**
214 citations	0.85%	121 sources
1,112 citations	5.25%	436 sources
2,448 citations	14.95%	815 sources
2,284 citations	24.00%	814 sources
2,102 citations	32.33%	754 sources
1,683 citations	38.99%	636 sources
1,270 citations	44.03%	519 sources
1,356 citations	49.40%	573 sources
1,426 citations	55.05%	572 sources
1,570 citations	61.27%	661 sources
Previous years: 9,777 citations		