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Original Article

Ferroptosis synergistically sensitizes wee1 inhibitors: a bibliometric study

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Abstract: Synthetic lethality (SL) is a lethal phenomenon with an important role in cancer treatment. This study was conducted to analyze the hotspots and frontiers in SL research. The Web of Science Core Collection (WOSCC) was used to identify the 100 top-cited articles related to SL research. Additionally, wee1 inhibitors combined with erastin were used to determine the effectiveness of SL *in vitro* and *in vivo*. Relevant articles were published mainly from 2009 to 2021, reaching a peak in 2020; articles published in 2010 were most frequently cited among the 100-top cited papers. Most studies (54%) were performed in the United States. Articles in *Nature Chemical Biology* were cited more frequently than articles in other journals, whereas *Nature* published the largest number of reports on SL. Among the 88 corresponding authors, CJ Lord was the most productive. Overlay visualization of keyword analysis revealed that the hotspots in SL research were PARP inhibitors, BRCA mutations, DNA damage repair, and carcinogenesis. CRISPR, ferroptosis, wee1, double-strand break (dsb) repair, myc, immunotherapy, and replication stress are emerging topics in SL research, whereas ovarian cancer, prostate stress, acute myeloid leukemia, and other topics have been used as disease models in recent years. The application and therapeutic strategy of SL in cancer is an emerging trend. Significantly, experiments verified that the wee1 inhibitor AZD1775 and ferroptosis activator erastin have synergistic effects on ovarian cancer *in vitro* and *in vivo*. Combining bibliometric analysis with experimental verification is a useful approach for SL research.

Keywords: Bibliometric analysis, synthetic lethality, PARP inhibitor, wee1 inhibitor, ferroptosis

Introduction

The concept of synthetic lethality was developed based on genetic studies of *Drosophila* in 1946 [1-4] and describes the incompatibility between pairs of alleles, with the co-occurrence of multiple gene mutations resulting in cell death [5]. Cancer has become one of the most common diseases as of the last century. SL is a promising approach for improving cancer research and treatment because of its higher effectiveness and safety compared to anticancer chemotherapy [6]. Unlike conventional targeted therapies, synthetic lethal therapies promote indirect mutation targeting by identifying an alternative synthetic lethal target, ranging from oncogenes to tumor suppressors, DNA repair, cancer metabolism, and even genetic background [7]. Therefore, synthetic lethal interactions may broaden anti-cancer treatment strategies and stimulate drug discovery.

Evaluative bibliometrics is a quantitative science that mainly involves citation analysis to filter out several useful articles [8]. This approach has been utilized in various fields, such as signaling pathway analysis [9], single gene analysis [10, 11], specific cell phenotype analysis [12], and disease analysis [13-17]. Unlike other methods used to systemically review a research field, bibliometrics can be used to not only analyze the general information and knowledge base but also evaluate the hotspots; studies of emerging research focus on a field of scientific research based on citation analysis [18, 19]. In recent years, bibliometrics analyses of cancer treatment methods have become increasingly important for evalu-

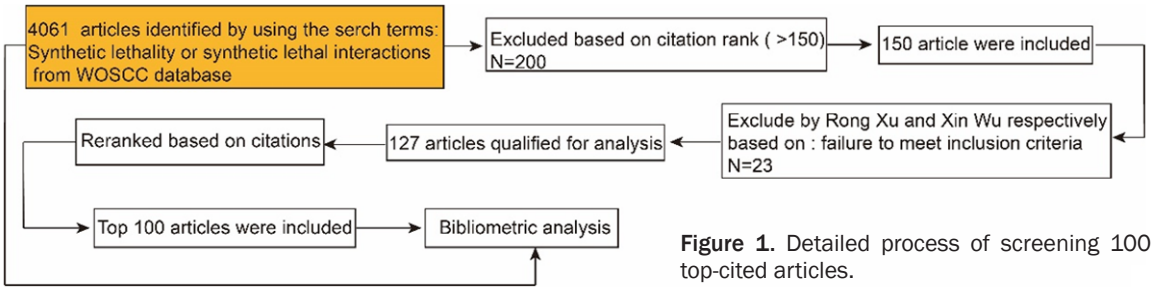


Figure 1. Detailed process of screening 100 top-cited articles.

ating research trends and formulating guidelines [9, 20]. Analysis of the top 100 most-cited articles in a research field may improve the understanding of how a discipline is developing and indicate future research directions [21].

In this study, we analyzed the top 100 most-cited articles combined with all articles in the SL research field under retrieval criteria based on bibliometric data. The detailed retrieval process is shown in **Figure 1**. We used bibliometric tools to objectively clarify the knowledge domain and future direction of SL research from the following perspectives. (1) Annual publications revealed growing trends in analysis of all articles. (2) We identified general information in SL research, such as cooperation information and individual influence in a research field, by analyzing productivity in different countries, institutions, journals, and authors. (3) We determined the knowledge structure, hotspots, and emerging trends by analyzing references with highest citation/co-citation reference burstness in the 100 top-cited articles, and performed keyword analysis of whole articles. (4) Finally, we experimentally verified our observations. Our results may provide new strategies for studying SL.

Materials and methods

Study design

The following information was listed for all 100 articles: date of publication, journal, corresponding author, first author, country, and institution. Citespace 5.8 was used to analyze the topic distribution of the published journals, and VOSviewer 1.6.17 was used to analyze hot topics and recent research trends. ClinicalTrials.gov was used to analyze clinical trials in which wee1 inhibitors were used for cancer treatment.

Search criteria

Data were extracted from WOSCC. "Synthetic lethality" and "synthetic lethal interactions" were used as search terms. The search was conducted on June, 14, 2022, and yielded 4,073 results. Articles published in 2022 were not included.

Data preprocessing

Two well-trained researchers reviewed the top 200 papers and performed screening to eliminate publications that did not meet the search strategy, as well as those that had been withdrawn. The papers were sorted by citation, and the following information was extracted from the top 100 papers: title, first author, corresponding author, journal, year of publication, country, institution, total citations, type of papers, categories, and citations in the last five years. The H-index of the authors was retrieved from the WOSCC. Information on the country and institution was extracted based on the affiliation of the corresponding author. For papers with multiple corresponding authors, the last author was chosen for analysis. The citation density was calculated as previously described [22]. We analyzed 4,073 papers using VOSviewer to detect hotspots and research trends in SL.

Cell culture and cell proliferation detection

Cavo-3 and SK-OV-3 cells were obtained from Central South University (Changsha, China). All cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) (BI biological industries, Israel) at a density of 5×10^3 in an incubator with 5% CO_2 at 37°C. The cells were added to 96- or 6-well plates and then treated with dimethyl sulfoxide (DMSO), AZD1775 (0.05

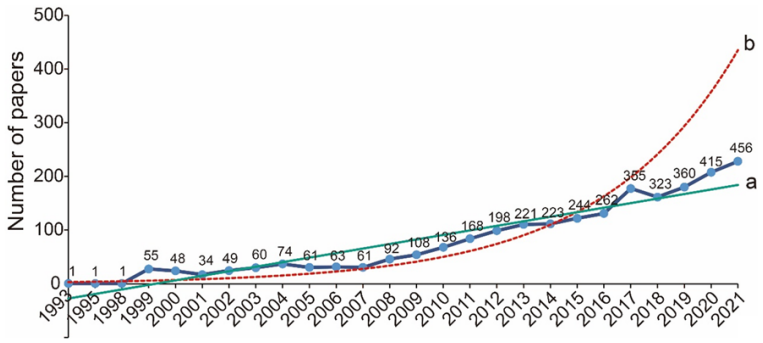


Figure 2. Annual output of SL research. Notes: SL was in its "germination stage" before 1999; 1999-2007 at "stable growth" stage; SL was in its "rapid development" stage after 2008. Linear adjustment (a): y = 16.938x-72.16, R^2 = 0.9014 (P < 0.001). Exponential adjustment (b): y = 5.2594$e^{0.1964x}$ 50.9082, R^2 = 0.9082.

μM), erastin (6 μM), or AZD1775 combined with erastin for 24 h after cell adhesion. AZD1775 was purchased from Merck Sharp & Dohme Corp., and erastin was provided by Selleck (Shanghai, China). For the CCK8 assay, 20 μL CCK8 reagent was added to the cells and incubated for 3 h. The OD value was measured using a microplate reader at a wavelength of 450 nm. For the clonogenic survival assay, the cells were cultured for two weeks. Cells were stained with 0.1% crystal violet dissolved in 95% ethanol. Clones containing more than 50 cells were counted. Cell proliferation was analyzed as described previously [23].

In vivo antitumor efficacy evaluation

Female 4-week-old BALB/c nude mice were purchased from the Animal Department of Central South University. Cavo-3 cells (5 × 10^6 cells) were injected into the backs of mice. AZD1775 (50 mg/kg) [24], erastin (30 mg/kg) [25], and saline treatment was started when tumors reached 90 to 110 mm^3 and continued for 5 days [26]. The tumor volumes and weights were measured. Tumor volumes were equal by (π/6) × AB^2 [27]. This research was approved by the animal ethics committee of Central South University.

Immunofluorescence

Immunofluorescence experiments were performed as previously described [28]. Paraffin sections were dewaxed and treated with absolute alcohol, 95% alcohol, and 75% alcohol. The cells were washed three times with phosphate-buffered saline, permeabilized in 0.5% Triton X-100 and blocked in 5% bovine serum albumin. The cells were incubated with a Ki67 monoclonal antibody (Invitrogen, Carlsbad, CA, USA) for 35 min at 37°C. Goat anti-rabbit IgG (H+L) cross-adsorbed secondary antibodies were used to detect the primary antibodies. DAPI was used to stain the cell nuclei.

Statistical analysis

Mann-Kendall trend test was used for time-dependent trend analysis. Spearman or Pearson's tests were used for correlation analysis. The *t*-test was used to statistically analyze the two groups. Statistical significance was set at P < 0.05. Data were visualized using GraphPad Prism 7 software (GraphPad, Inc., La Jolla, CA, USA) and Microsoft Office Excel 2016 software (Redmond, WA, USA).

The bibliometric software Citespace and VOSviewer were utilized for this article [29]. The dual-map overlay of journals was adopted to analysis the subject distribution of academic journals. Network/overlay/density visualization of keywords was applied to discovery important keywords, statistics frequency and cluster keywords. The size of nodes in network visualization represents occurrences. Moreover, time-dependent overlay visualization provides keywords that change over time, which are shown in color from blue to yellow. The data processing was completed by three researchers.

Results

Annual growth trend of total papers (n = 4061)

A total of 4061 publications was extracted from the WOSCC database. To visualize growth trends in SL research, data were obtained from the WOSCC. As shown in **Figure 2**, the number of SL-related papers steadily increased in 1999-2008 and increased exponentially after 2009, and reach the peak in 2021. To determine whether the growth of papers on SL conformed to Price's law, linear and exponential adjustments were performed on the acquired

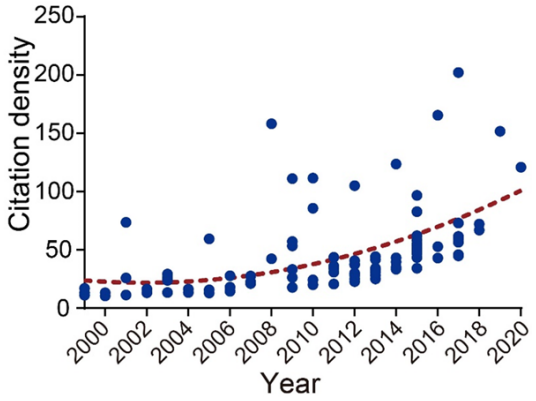


Figure 3. Time-dependent citation density trend of 100 top-cited articles in SL research. Mann-Kendall trend test showed an increasing trend between the citation density and the time (P = 3.445E-9). Notes: citation density was calculated by "citation without self-citation/paper age".

data. The equation y = 16.938x-72.16 (correlation coefficient = 0.9014) showed a linear fit. Another equation, $y = 5.2594e^{0.1964x}$ (correlation coefficient = 0.9082), was obtained from the exponential curve.

Time analysis (n = 100)

Of the top 100 articles, 71% were published after 2010. Articles published prior to 2020 accounted for 29%, and those published since 2015 only accounted for 18.3% (Figure S1). The trend in citations over time was not significant (P = 0.219), with the citation density showing an increasing tendency over time (P = 3.445E-9) (**Figure 3**). A positive correlation was observed between time and the citation density (Spearman's r = 0.639, P < 0.01). Among the top 100 cited papers, there were 57 original articles and 43 articles (including reviews and others); the number of citations ranged from 191 to 2215. The largest number of papers was published from 2009 to 2015 (**Figure 4A**), which indicates that scholars may have made better progress in the study of SL from 2009 to 2015. Moreover, there were citation bursts in 2001 and 2008 (**Figure 4B**), indicating that SL was widely studied in these years. The 10 most cited articles on SL are listed in **Table 1**, the 10 most citation density articles on SL are listed in **Table 2**; the top 10 cited articles in the last five years are listed in **Table 3**. The overlapped data in **Tables 1-3** was showed in (Figure S2), indicating that these four articles were important in SL research.

Field categories and journal analysis (n = 100)

The top 100 most-cited papers mainly focused on the fields of biochemistry and molecular biology (34%), followed by oncology (24%), science technology (14%), and general and internal medicine (4%) (Figure S3). The largest number of citations was of articles published in *Nature Chemical Biology*, followed by those in *Science and Nature*; publication in these important journals indicates that the findings reported in these articles were impactful to the field. *Nature* published the largest number of SL-related papers, with seven articles among the top 100 most cited papers (**Figure 5A**). The journal's dual-map overlay represents the topic distribution of the academic journals (**Figure 5B**); the papers on SL research published in journals of MOLECULAR, BIOLOGY, IMMUNOLOGY mainly cited by journals in MOLECULAR, BIOLOGY, GENETIC. The top 100 articles were published in 55 journals; the top 10 journals with the largest number of cited articles are listed in **Table 4** and are all JCR (Journal Citation Report) 1 divisions with impact factors of over 10.

Country and institutional analysis (n = 100)

The country distribution of the articles ($n \geq 2$) is shown in **Figure 6A**. Studies were conducted in 14 countries, with the largest number in the USA (n = 54), followed by the UK (n = 15). The UK had the largest citation density, reflecting the strong influence of researchers in this field. Contributions from different countries can also be evaluated based on the involvement of at least one author from a country in highly cited articles. We analyzed the number of articles published in these countries, percentage of total articles published, percentage of articles published by individual countries, percentage of articles published by international collaborations, percentage of articles published by the first authors, and percentage of articles published by the corresponding authors. The percentage of independent articles of the total number of highly cited articles in each country was evaluated. The USA had the largest number of articles by individual country (41%), partner country (18%), first author (54%), and corresponding author (54%) (**Table 5**). Globally, the top 100 cited articles were from 71 research institutions, among which 12 published two or more papers. Authors from Harvard University

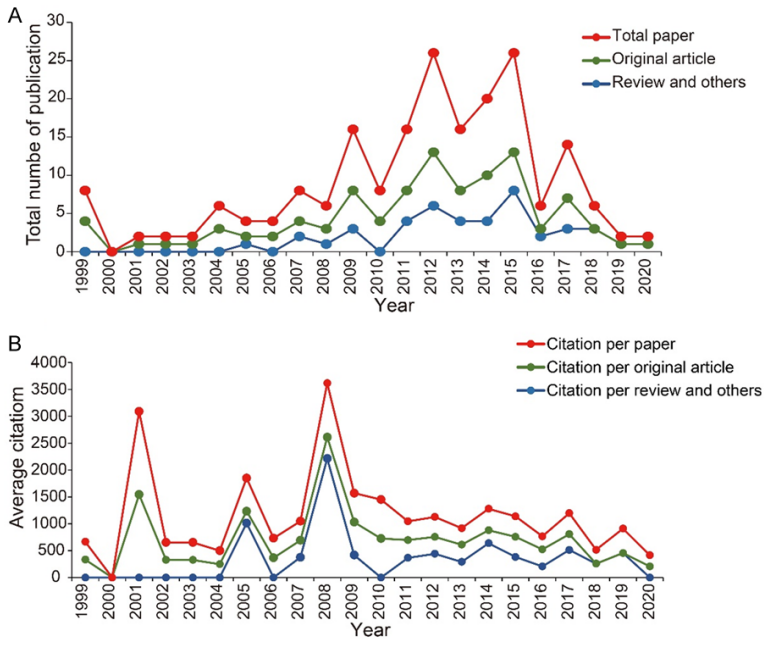


Figure 4. Number of publication and citation analysis of 100 top-cited articles in SL research. A. Trend of the number of total papers, original articles, and review or others in 1999-2020. A majority of articles including original articles and other type of articles were published from 2009 to 2015 (64%, n = 64). B. Trend of the citation of total papers, original articles, and reviews or others from 1999-2020. Articles with most average citations were published in 2001 (Average citation = 1545) and 2008 (Average citation = 1003.33).

published the largest number of articles (n = 6), whereas University of Toronto obtained largest citations per papers, with an average of 980 citations (**Figure 6B**).

Author analysis (n = 100)

Moreover, authors with at least two or more articles are shown in **Table 6**, along with the number of articles, citations, and H-index. The total number of citations and H-index reflects the influence of a researcher in a specific field. Eighty-eight corresponding authors were included in the top 100 cited articles; nine authors published articles in at least two or more journals (**Figure 7**). Among the nine authors, CJ Lord published the largest number of articles (n = 4), had the largest number of total citations (2658), and had the highest H-index (66).

Top 10 citation reference and reference burst analysis

The influence of published in a specific time on citation analysis provides quantitative information that is helpful for further research. We analyzed the effect of time on citations for the top ten cited references. As showed in Figure S4, since year 2020, Lord CJ, et al. was cited more times than other top-cited articles, although the article was published in 2017. The second-most cited article was by Hopkins AL published in 2008. A reference burst occurs when references are cited frequently in a period of time [30]. As shown in **Figure 8**, of the references, 32% (8/25) appeared to have a citation burst begin in 2010, followed by in 2009 and 2013 (16%, 4/25). The paper entitled "Inhibition of poly (ADP-ribose) polymerase in tumors from BRAC mutation carriers" [31] had the largest burst length of 2.73 from 2010 to 2020 and was published by PC Fong in 2009.

Trends in research subtopics

VOSviewer was used to perform co-occurrence keyword analysis (**Figure 9**). A total of 5,134 keywords was extracted, with 216 terms appearing more than five times. The cluster map shows the knowledge structure of the research field, and the overlay indicates hotspot keywords in this field. The density map of keywords reveals keywords that co-occur with high frequency, also revealing hotspots in the field [30]. As shown in **Figure 9A**, PARP inhibitors were the most important keywords, with 240 co-occurrences in three clusters, followed by DNA damage repair, BRAC mutations, and carcinogenesis. Cluster 1 (shown in red) was the largest cluster, which contained 80 terms, including carcinogenesis, cancer therapy, apoptosis, target therapy, biomarkers, and drug resistance. Cluster 1 mainly included terms related to the functions of SL. Cluster 2 (shown in green) contained terms related to drug development for cancer therapy based on SL and included 65 items, such as DNA damage repair, genetic interactions, RNA interference, yeast, and *Saccharomyces cerevisiae*. Cluster 3 (shown in blue) was focused on the application

Table 1. Top 10 articles with largest citation density

Title	Type of article	Citation rank	Citation density	Year
PARP inhibitors: synthetic lethality in the clinic	Review	8	195.4	2017
Network pharmacology: the next paradigm in drug discovery	Review	1	158.2	2008
A view on drug resistance in cancer	Review	18	151.7	2019
Causes and consequences of replication stress	Review	9	122.6	2014
Drugging the undruggable RAS: mission possible	Review	10	118.1	2014
Oral poly (ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: a proof-of-concept trial	Article	4	107.1	2010
Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1	Article	3	105.1	2009
The DNA damage response and cancer therapy	Review	5	103.3	2012
The regulation and functions of DNA and RNA G-quadruplexes	Review	92	100.5	2020
Targeting the DNA Damage Response in Cancer	Review	13	95.3	2015

Table 2. Top 10 articles with largest citation in last 5 years

Title	Type of article	Citation rank	5 years citation	Year
PARP inhibitors: synthetic lethality in the clinic	Review	8	1011	2017
The DNA damage response and cancer therapy	Review	5	962	2012
A view on drug resistance in cancer	Review	18	860	2019
Targeting the DNA damage response in cancer	Review	13	805	2015
The regulation and functions of DNA and RNA G-quadruplexes	Review	92	749	2020
Molecular alterations in triple-negative breast cancer-the road to new treatment strategies	Article	40	609	2017
Drugging the undruggable RAS: mission possible	Review	10	583	2014
Systematic genetic analysis with ordered arrays of yeast deletion mutants	Article	2	523	2001
Causes and consequences of replication stress	Review	9	493	2014
Targeting oncogenic Myc as a strategy for cancer treatment	Review	64	455	2018

Table 3. Top 10 cited articles without self-citation

Title	Type of article	Citation rank	citation	Year
Network pharmacology: the next paradigm in drug discovery	Review	1	2215	2008
Systematic genetic analysis with ordered arrays of yeast deletion mutants	Article	2	1545	2001
Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1	Article	3	1444	2009
Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: a proof-of-concept trial	Article	4	1339	2010
The DNA damage response and cancer therapy	Review	5	1051	2012
Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer: a proof-of-concept trial	Article	6	1030	2010
The concept of synthetic lethality in the context of anticancer therapy	Review	7	1012	2005
PARP inhibitors: synthetic lethality in the clinic	Review	8	1011	2017
Causes and consequences of replication stress	Review	9	989	2014
Drugging the undruggable RAS: mission possible	Review	10	982	2014

of SL and contained 60 terms, such as BRAC mutations, ovarian cancer, breast cancer, atm, DNA damage response, triple-negative breast cancer, and clinical trials. As shown in **Figure**

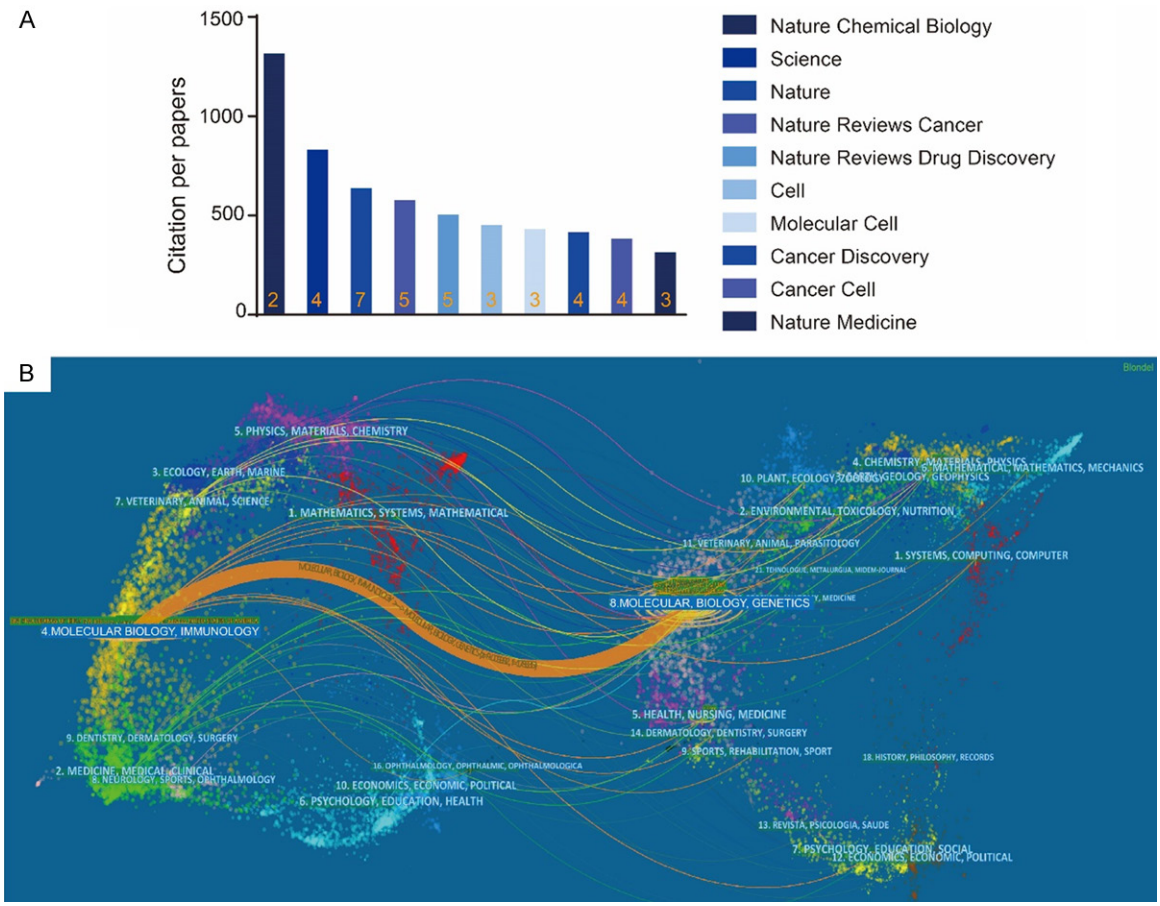


Figure 5. Journal analysis in 100 top-cited articles in SL research. A. Distribution of journals with 2 or more articles, and number of articles were showed within bottom of bar. B. Citespace was used for dual-map overlay journals analysis. Notes: the path colored with orange represents the citation relationship between citing journals (left) and cited journals (right).

Table 4. 10 countries of top-cited articles

Country	TRP	SPR	CPR	FPR	RPR	S
USA	54%	41%	18%	54%	53%	75.9%
UK	15%	12%	12%	16%	17%	80%
Germany	7%	5%	6%	8%	7%	71.4%
Spain	5%	3%	4%	5%	5%	60%
Canada	5%	5%	3%	5%	5%	100%
Japan	3%	3%	1%	3%	3%	100%
Netherlands	2%	1%	2%	45	2%	50%
Sweden	2%	N/A	2%	1%	1%	0
Austria	2%	N/A	N/A	N/A	2%	0
Italy	1%	N/A	1%	1%	1%	0
Norway	1%	N/A	1%	N/A	1%	0
Slovakia	1%	N/A	1%	1%	1%	0
Singapore	1	N/A	1%	1%	1%	0
China	1%	1%	1%	2%	1%	100%

TP: total number of articles; TPR (%): the percentage of total articles; SPR (%): articles with single country; CPR (%): internationally collaborative articles; FPR (%): first author articles; RPR (%): corresponding author articles in their total articles; S% present the percentage of single country articles in total articles for each country; N/A means not available.

9B, each node was colored according to the time at which it occurs, forming a blue to yellow gradient. Immunotherapy, wee1, ovarian cancer, ferroptosis, triple-negative breast cancer, CRISPR, combination therapy, and precision medicine are emerging keywords that represent new research directions. Furthermore, most of the yellow nodes appeared in Cluster 3, demonstrating that researchers have focused on the clinical application of SL. The density map of the keywords shows keywords co-occurring with high frequency. As shown in **Figure 9C**, these terms included PARP inhibitors (4.6%), DNA damage repair (3.09%), BRAC mutations (2.9%), carcinogenesis (2.8%), homologous recombination (1.6%), target therapy (1.6%), ovarian cancer (1.4%), breast cancer (1.3%),

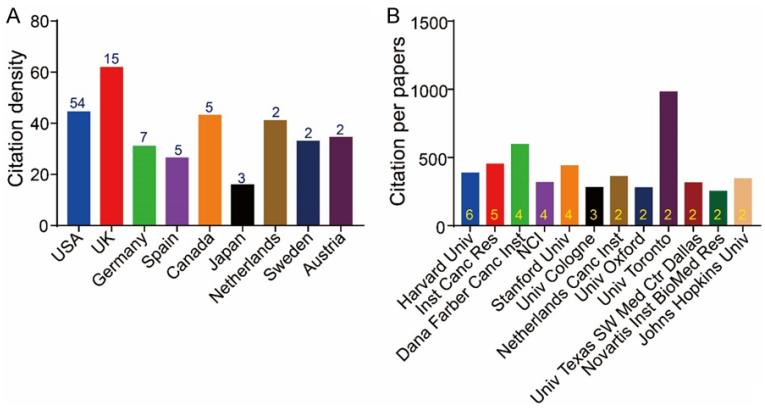


Figure 6. Countries and institutions analysis of 100 top-cited articles in SL; A. Citation density analysis based on countries revealed that USA is the most productive countries in 100 top-cited articles, while UK has the largest citation density. Notes: the citation density of countries was equal to "citation without self-citation/paper age". B. Analysis of mean citation and publication number of institution. Harvard University is the most productive institution in 100 top-cited articles, while University of Toronto ha largest average citation number.

DNA damage response (1.3%), and cancer therapy (1.28%). Among the top 10 co-occurring keywords, half of the terms were from Cluster 3.

Ferroptosis synergistically sensitizes wee1 inhibitors to ovarian cancer

PARP inhibitors were the first listed synthetic lethal drugs for cancer treatment. Since then, other inhibitors, such as RAD5A, wee1, and PRMT5, have entered the clinical trial stage. Wee1 was one of the most common keywords in recent years according to time-dependent occurrence keyword analysis. Analysis of the current status of clinical trials of wee1 inhibitors revealed 51 clinical studies of the effects of the wee1 inhibitor on cancer in the Clinical Trials Database on June 6, 2022, https://ClinicalTrials.gov/. The largest number of clinical trials was performed in North America, with 18 trials in the United States. Among them, AZD1775 has been used in 16 institutions, followed by MK-1775 in five clinical trials (Figure S5A). The time-dependent overlay map revealed that ovarian cancer was the most common cancer, with most recent research employing synthetic lethal drugs. We identified five studies of ovarian cancer based on wee1 inhibitors, most of which were performed in the United States (Figure S5B). Ferroptosis is an emerging topic in synthetic lethal research. PARP inhibitors can promote ferroptosis and act synergistically with ferroptosis inducers in ovarian cancer treatment [32]. In this study, we treated ovarian cancer cells with a wee1 inhibitor (AZD1775) and ferroptosis inducer (erastin). As shown in **Figure 10A** and **10B**, erastin and AZD1775 showed stronger inhibition of ovarian cancer cell viability compared to the effects of AZD1775. Moreover, colony formation assays showed that compared with the control groups, treatment with erastin and AZD1775 more strongly inhibited cell proliferation (**Figure 10C**). To verify whether ferroptosis affects the efficacy of AZD1775, we transplanted a tumor mouse model with SK-OV-3 cells and then treated the mice with AZD1775, erastin, or both (**Figure 10D**). As predicted, AZD1775 or erastin alone reduced cell growth; combination treatment with AZD1775 and erastin reduced the tumor size more rapidly, showing synergistic effects (**Figure 10E**, **10F**). As showed in **Figure 10G**, the synergistic inhibition of wee1 by AZD1775 in combination with erastin could significantly inhibit expression of Ki67. These results suggest that erastin can synergistically increase therapeutic efficacy in ovarian cancer.

Discussion

Bibliometric analyses are useful for evaluating widely researched topics or cited journals, authors, countries, institutions, and references in a research field. These studies have been performed in several disciplines [1, 33-36]. Here we performed bibliometric analysis of SL. This research field is more prominent in the USA and UK than in other countries, with articles typically published after 2008. Most articles were original articles. The most evaluated topics in the original studies were DNA damage repair, BRAC mutations, and carcinogenesis. The emerging trend of this research is therapy in ovarian cancer, prostate cancer, and combined CRISPR or ferroptosis with SL to discover new genetic interactions or potential therapeutic targets.

Table 5. Authors with two or more articles

First Author	Institutions	Rank of Paper	Total Citations	Number of Papers	H-index
Lord CJ	Inst Canc Res, UK	6, 9, 59, 61	2658	4	66
Osman C	University of Cologne, Germany	29, 78, 90	832	3	11
Pan XW	Johns Hopkins Univ, USA	20, 76	685	2	28
McMillin DW	Dana Farber Canc Inst, USA	55, 75	549	2	24
Chan DA	Stanford Univ, USA	46, 99	537	2	29

Table 6. Top10 journals with largest citation

Journal	IF (2021)	number	Citations Average	Country	JCR division
Nature Chemical Biology	15.040	2	1305	USA	Q1
Science	47.728	5	821.25	USA	Q1
Nature	49.962	7	628.7143	UK	Q1
Nature Reviews Cancer	60.716	5	569.8	UK	Q1
Nature Reviews Drug Discovery	84.694	3	495.6667	UK	Q1
Cell	41.582	5	441.8	USA	Q1
Molecular Cell	17.87	3	421.3333	USA	Q1
Cancer Discovery	39.39	4	408.25	USA	Q1
Cancer Cell	31.742	4	374.75	USA	Q1
Nature Medicine	53.440	3	305	USA	Q1

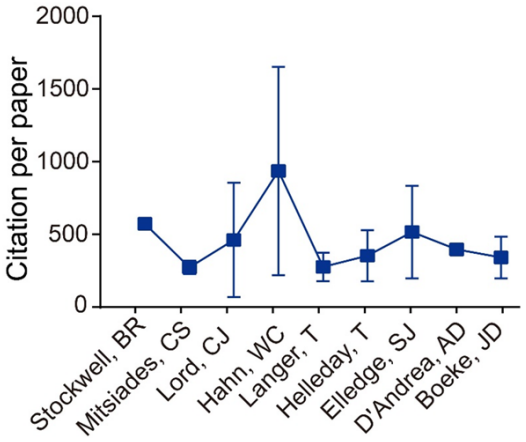


Figure 7. Author analysis of 100 top-cited articles in SL; Mean citations based on corresponding authors with 2 or more articles. CJ Lord CJ obtained the most papers, while WC Hahn, had the largest average citation number.

Bibliometric findings

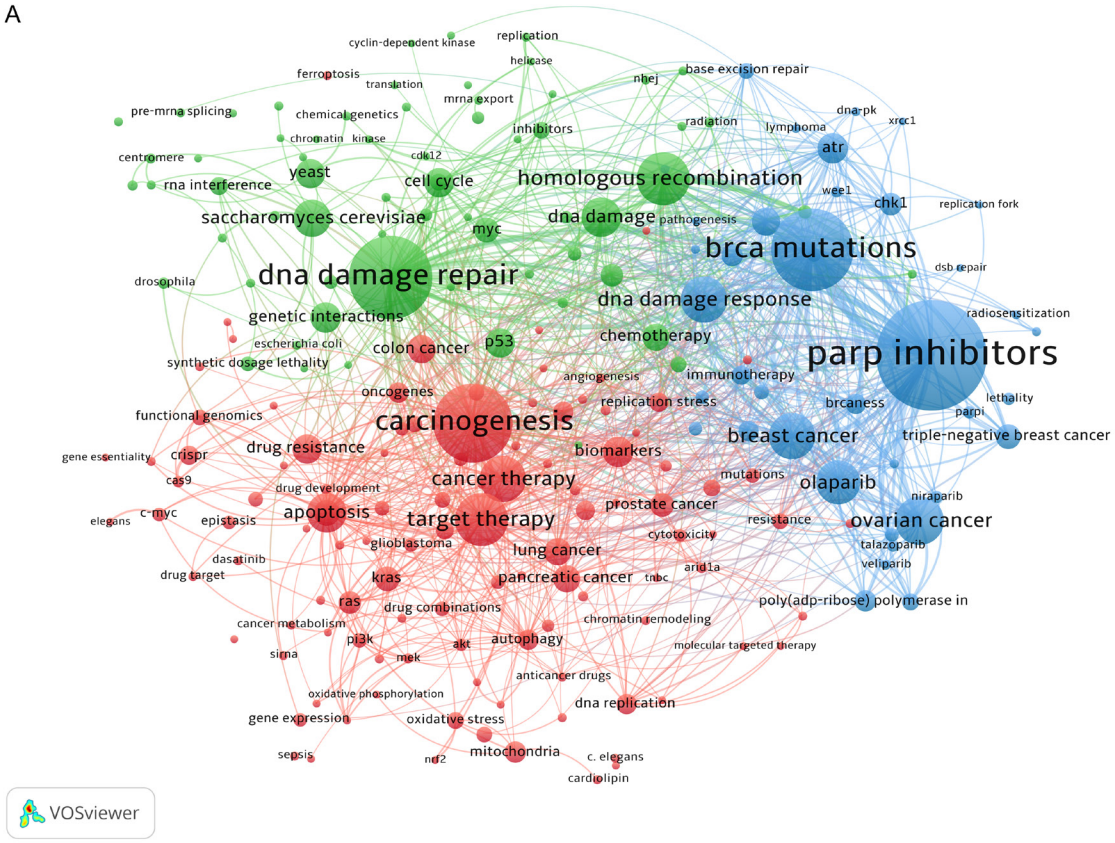
The concept of SL originatd from the preliminary observations of the American geneticist Calvin Bridges approximately 100 years ago, who noted that when crossing fruit flies, the genetic combination of specific mutant gene pairs is fatal, despite that inheritance of either mutant gene alone led to completely normal viability [36]. Functional genomic screening based on the concept of SL can be used to identify drug targets [37]. Based on our data from the WOSCC database, the top 100 articles related to SL research were published in 55 journals by 88 authors from 71 institutions in 14 countries. The number of publications on SL research surged in 2008 and peaked in 2021. The linear tendency and exponential trend of publications on SL indicated a linear growth pattern of these publications. However, the distribution of publications did not always comply with Price's law because of several complex factors, such as the economy of a country, government investment, and new discovery. The linear tendency of publications on SL showed that the burst period of research has not been reached. Thus, further studies are needed in this field. These growth trends will continue based on the citation density diagram of the growth curve.

Globally, the geographical distribution of studies on SL was unbalanced. We found that 54% of the top 100 cited publications were came from the USA, followed by the UK. Interestingly, among the 10 countries with high citation densities, 60% were from Europe. This result may be explained as follows: (1) The concept of SL

Figure 8. The top 25 references with strongest citation bursts. Notes: Citation burst is presented in red bars, while publication time is presented in blue bars. Article published by PC Fong in 2009 has strongest amount of bursts.

originated in the USA by the geneticist Calvin Bridge, whereas Dobzhansky Theodore, who was also from the USA, proposed SL in 1946. Scientists from the Fred Hutchinson Cancer Research Center suggested that SL can be used in cancer therapy [38, 39], and foundational research on SL was performed in the USA. (2) Studies of DNA damage repair, cancer, and clinical transformation in the USA has promoted further development of SL research. Therefore, researchers in the USA began studying SL earlier and more thoroughly. (3) Various

A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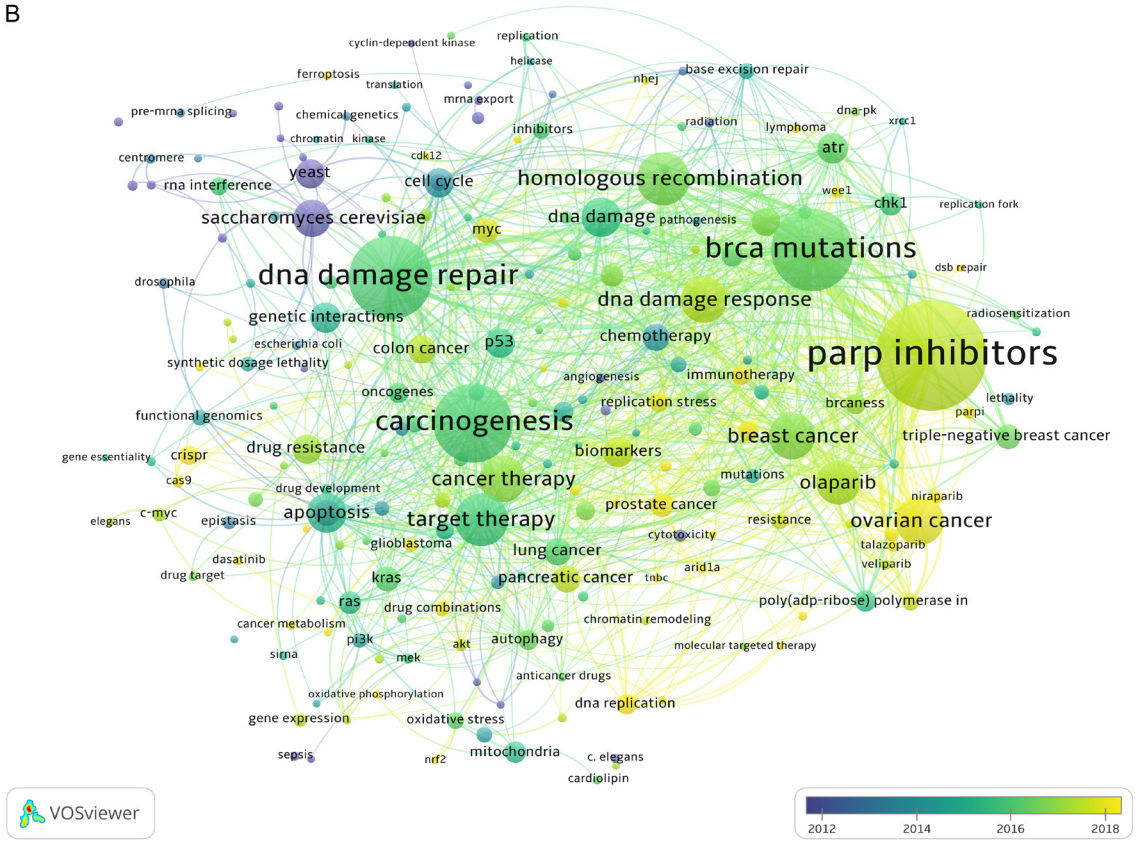
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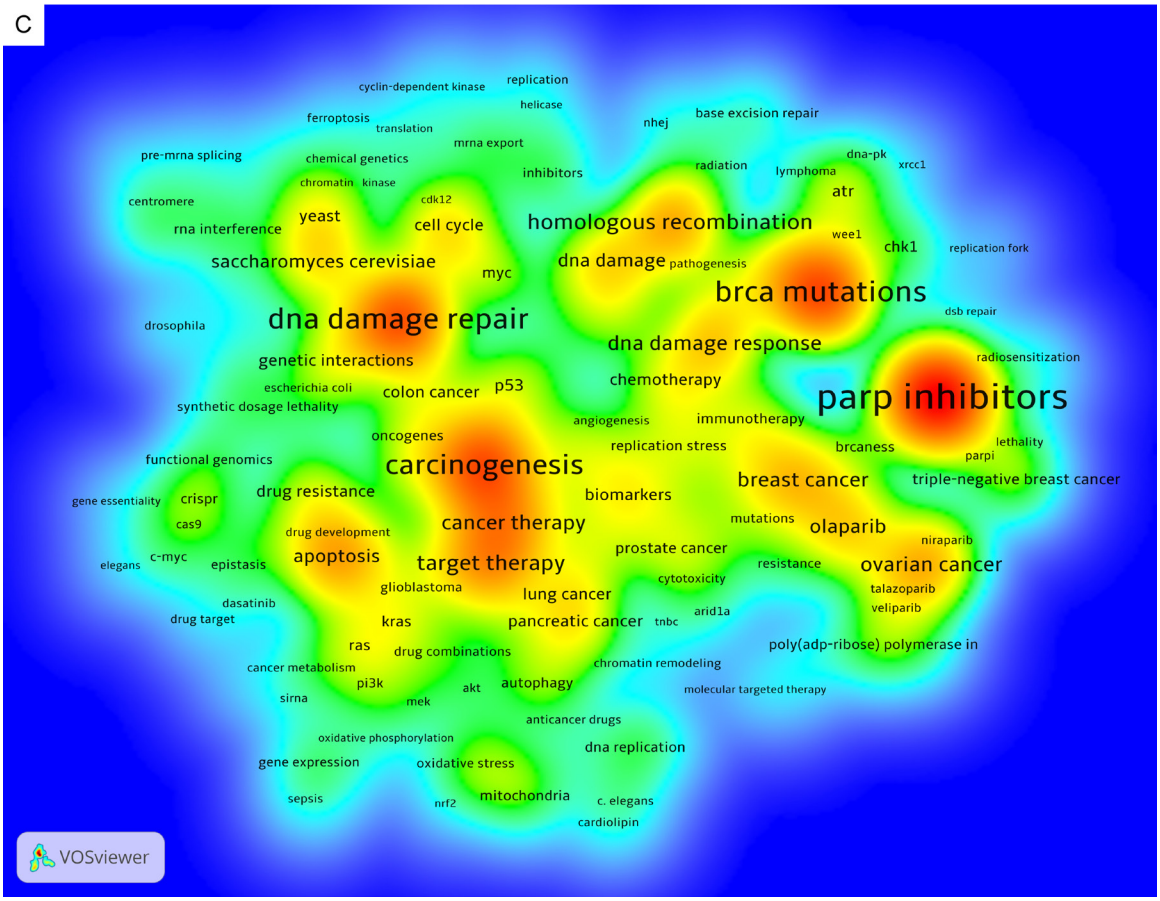

Figure 9. Trending topics in SL research. A. Keywords co-occurrence cluster. Notes: t > 5, contains 216 items, 3 clusters, the size of node and word present the co-occurrence frequencies. Anode in the same color is classified into a cluster. B. Overlay map. Notes: blue to yellow gradient shows the keywords changed over time. C. Density map. Notes: the size of keyword, size of node, and opacity of yellow are positively related to the co-occurrence frequency.

countries, including the USA, the Netherlands, and Germany, had an advanced economy to support basic medical research [40].

Researchers from Harvard University published the largest number of articles (n = 6). This world-renowned private research university also collaborated with universities worldwide. Although the University of Toronto had only two publications, the average number of citations was significantly higher than those of other institutions. The most prolific institution was Harvard University, although Toronto reported more influential work SL research. Highlighting the contributions of influential researchers can help identify potential collaborators, directions, and guidelines in a scientific field. In this study, CJ Lord published the largest number of papers among the top 100-cited articles, whereas Hahn WC had the largest average number of citations. The most prolific or outstanding contributing authors were from the UK, indicating that influential researchers were mainly from institutions with large nubmers of citations.

Nature, *Cell*, and *Science* are the most prolifically cited journals, emphasizing the importance of SL research, whereas *Nat Chem Bio*, with an average citation number of 1305, was the most influential journal in SL research. Both journals published articles related to cell biology, which is consistent with the results of discipline categories and dual-map analysis. SL-related studies remain focused on basic research. The average impact factor of the journals that published the top 10 cited articles were greater than ten, indicating that these journals have interests and play vital roles in SL-related research.

Research hotspots and emerging trends

Network analysis was performed to identify the knowledge structure and emerging trends

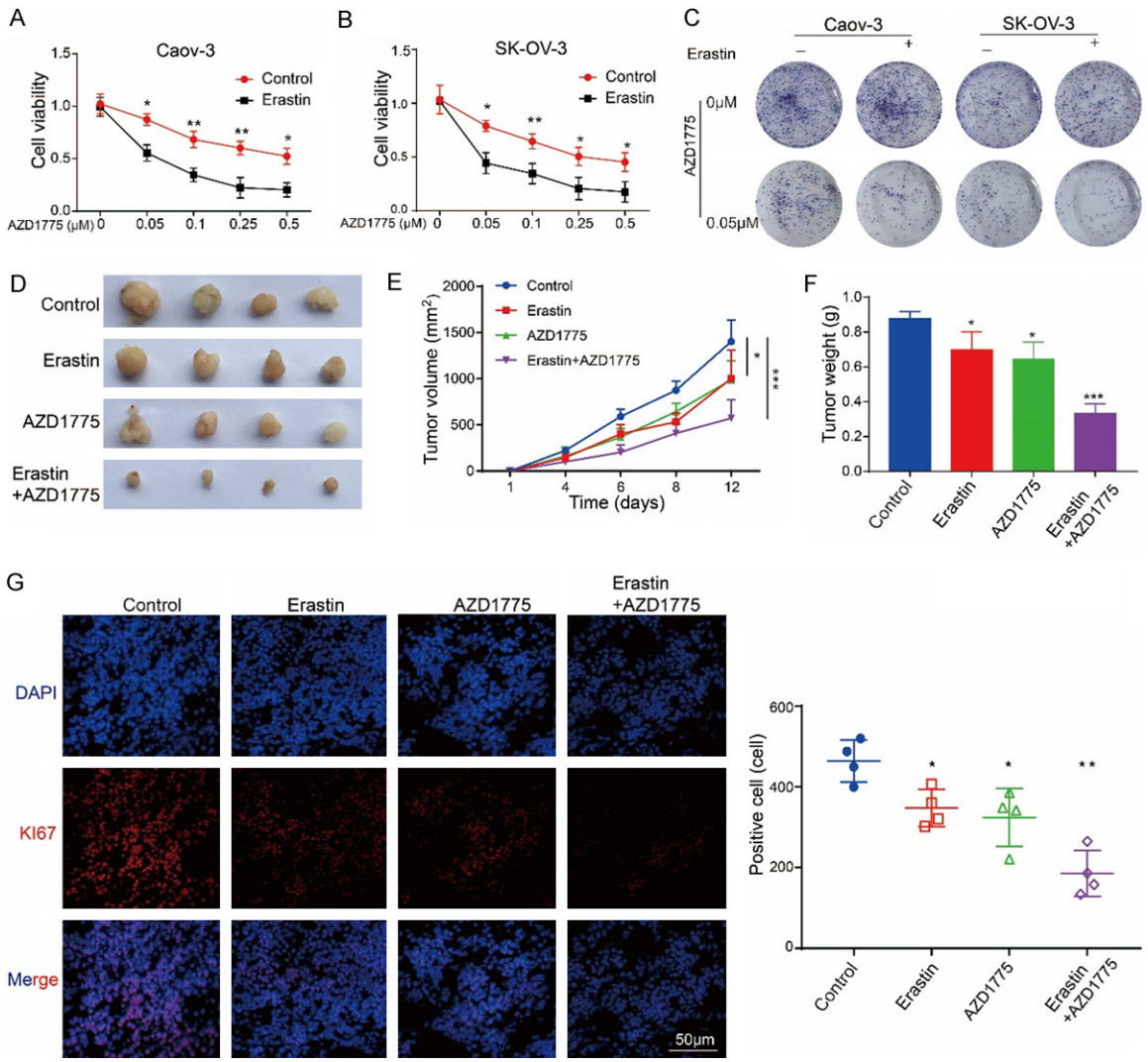

Figure 10. Wee1 inhibitor correlates with ferroptosis-mediated tumor growth in vitro and in vivo. A, B. Analysis of cell proliferation level in Caov-3 and SK-OV-3 cells treated with AZD1775 or erastin or AZD1775 in combination with erastin for 24 h. C. Representative images of clonogenic assay in Caov-3 and SK-OV-3 cells treated with AZD1775 or erastin or AZD1775 in combination with erastin. D. Representative images of SK-OV-3 xenograft tumors treated with ADZ1775 or erastin or AZD1775 in combination with erastin at experimental denpoints. E. Tumor volume was detected at different time points. F. Tumor weights were calculated at experimental endpoints. G. Immunofluorescence images of KI67 in different groups. Data are presented as mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001. *P* values were calculated by 2-tailed unpaired Student's t-test.

based on frequently used words and the year of occurrence in the list of keywords. Based on analysis of the frequency of keywords, terms such as PARP inhibitors, BRCA mutations, DNA damage repair, carcinogenesis, and target therapy are core research fields focused on cancer treatment, suggesting that SL is a research priority in cancer-related studies. PARP is a key molecule in DNA damage repair. PARP inhibitors can competitively combine PARP to result in the accumulation of a large amount of single-stranded DNA damage in cells with the BRCA1/2 mutation, eventually resulting in cancer cell death. PARP inhibitors have been approved for the treatment of refractory ovarian, breast, and pancreatic cancers. Although PARP inhibitors have been evaluated in preclinical studies, further research is needed, such as to determine the mechanism by which these inhibitors kill or inhibit tumor cells. Based on this mechanism, biomarkers for targeted treatment of patients with cancer and methods for

increasing the effectiveness of therapy can be developed. In addition, analysis of the effect of time on citations showed that the article entitled "PARP inhibitors: Synthetic lethality in the clinic [41]", published by CJ Lord in 2017, was the most cited SL-related article in 2021. This review describes current knowledge on PARP inhibitors and methods for improving clinical effectiveness (potential PARP inhibitor combined with chemotherapies/targeted agents/immunotherapies). Reference burst analysis showed that the original article "Inhibition of poly (ADP-ribose) polymerase in tumors from BRCA mutation carriers [31]" has the strongest burst. This phase 1 trial of olaparib showed that the drug has an acceptable side effect profile without the toxic effects commonly associated with conventional chemotherapy. Furthermore, the author suggested that designing molecularly targeted therapies is appropriate for some patients whose tumors have the same molecular defect but different origins. These findings accelerated the development of anticancer drugs. In summary, SL studies of PARP inhibitors in tumor therapy will continue to be performed, resulting in the development of anticancer drugs, such as wee1 inhibitors [5] ATR inhibitors [42], and PRMT5 inhibitors [43].

Overlay visualization focuses on research in the scientific field. Recent studies indicated that keywords such as ovarian cancer, immunotherapy, prostate cancer, wee1, PDL1, CRISPR, and ferroptosis have been widely used in recent years. Ferroptosis, proposed in 2012, is a type of non-apoptotic regulatory cell death induced by erastin. This process is closely related to SL, as the origin of ferroptosis was determined during SL screening of drugs sensitive to RAS mutant cells. Ferroptosis increases the sensitivity of cancer stem cells and tumors (undergoing epithelial-mesenchymal transition, resistance to anticancer drugs, or targeted therapy) to drug treatment [44]. Researchers showed that combination treatment with PARP inhibitors and ferroptosis activators synergistically promotes ferroptosis and sensitize BRCA-proficient ovarian cancer *in vitro* and *in vivo* [32]. Combining wee1 inhibitors with ATR inhibitors and immune checkpoint blockers (PDL1 inhibitors) shows potential for use in cancer therapy [45]. Therefore, we analyzed clinical trials of wee1 inhibitors in cancer and ovarian cancer, selecting the generally used inhibitor AZD1775 as a synergetic partner of ferroptosis for verification. AZD1775 acted synergistically with erastin to inhibit cell proliferation and tumor growth *in vitro* and *in vivo*.

In summary, we analyzed SL research and found that PARP inhibitors remain a hotspot. Based on this emerging trend, we first conducted *in vitro* and *in vivo* experiments to confirm the synergistic effect of the wee1 inhibitor and ferroptosis inducer as a promising therapeutic strategy for treating ovarian cancer.

Limitations

In this bibliometric study of SL research, we retrieved only the top 100 cited articles, which were the most representative articles. This study was based on the WOSCC, one of the most common databases used in bibliometric analysis. However, some publications are not included in the database. Additionally, the total citation, citation in the last five years, citation without self-citation, emerging keywords, impact factor of journals, and other factors change over time. Therefore, bibliometric studies can only provide references for a relatively short time period. Finally, we only evaluated the antitumor effects of wee1 inhibitor and erastin; their mechanisms require further research.

Conclusion

Based on bibliometric analysis of the 100 most-cited SL-related articles, we provide information on annual trends in publications; most productive or cited countries, institutions, journals, and authors; and list landmark publications in SL research. Furthermore, we summarized hotspots in SL research and suggest emerging trends in the near future. Bibliometric analysis can improve synthetic lethal strategies in cancer therapy.

Acknowledgements

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Disclosure of conflict of interest

None.

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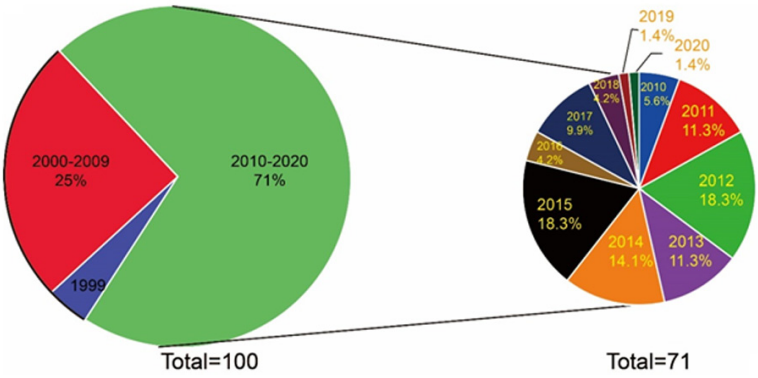

Figure S1. Time analysis of 100 top cited articles in SL research. A majority of articles were published in 2010-2020 (71%). Moreover, the largest number of articles were published in 2012 and 2018.

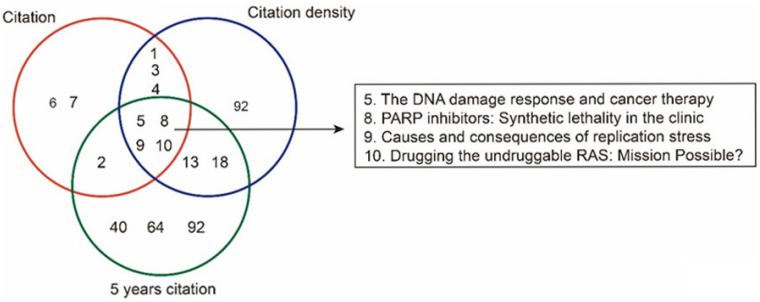

Figure S2. Venn diagram analysis of the 10 top cited articles with citations in total, citation density, and citations in latest five years. A total of 4 intersected articles were found by taking the intersection of the top cited articles from **Tables 1**-**3**.

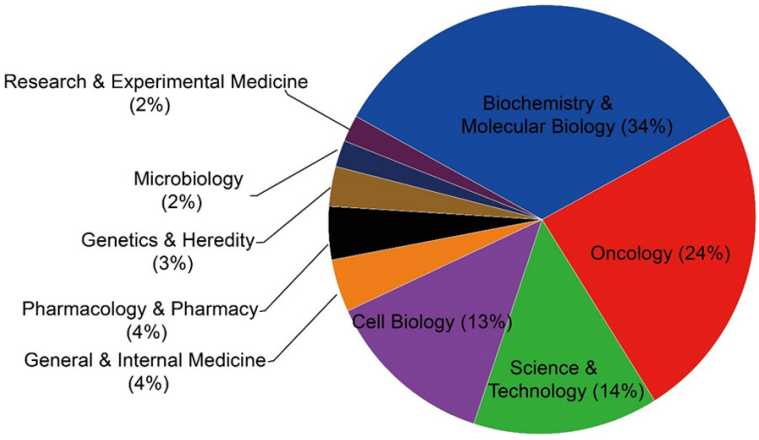

Figure S3. Discipline categories distribution of 100 top-cited articles in SL research. SL research focused on biochemistry & molecular biology, followed by oncology and science & technology.

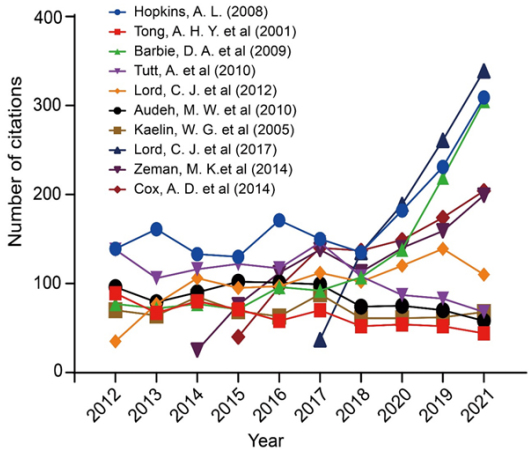

Figure S4. Effect of time on citation analysis of the 10 top-cited papers. Lord CJ published an article in 2017, which acquired the highest citation in 2021.

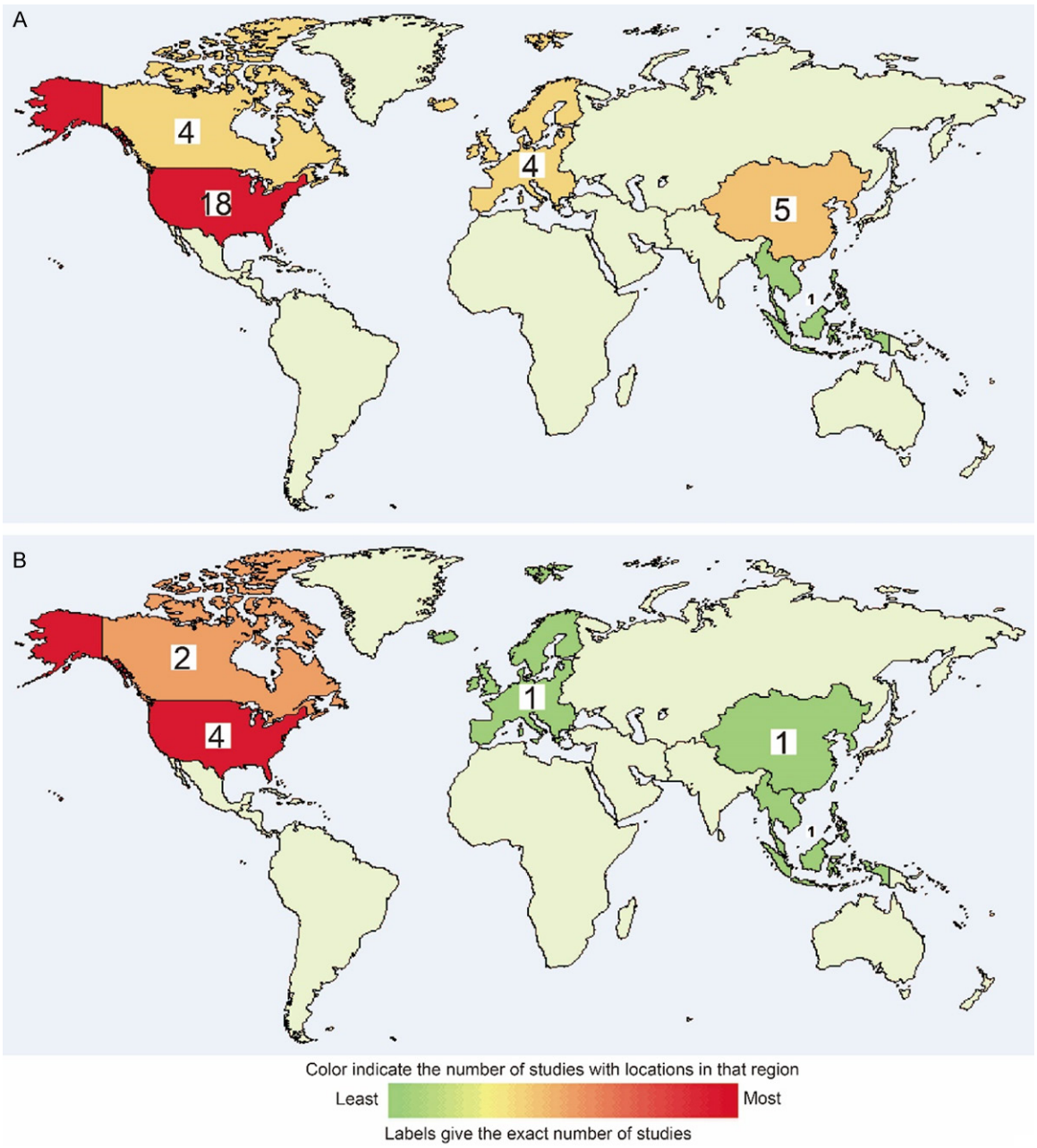

Figure S5. Clinical trial information of wee1 inhibitors in cancer treatment. A. Geographical distribution of clinical trials of wee1 inhibitors in cancer therapy. B. Geographical distribution of clinical trials of wee1 inhibitors in ovarian cancer therapy. Notes: Colors indicate the number of studies with locations in that region; the colors from green to red indicate fewest and most number of studies respectively.