DOI: https://doi.org/10.47391/JPMA.079

Clinically-targetable vulnerabilities in cancer metabolism: A systematic review and meta-analysis

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Abstract

Objectives: To investigate the efficacy and safety of targeting cancer metabolic vulnerabilities with specific anticancer agents.

Method: The systematic review and meta-analysis entailed search on PubMed, Embase and Google Scholar databases for cohort-based studies or clinical trials which reported hazard ratio for overall survival and/or median overall survival of patients treated with metabolically-active anticancer drugs. Data was analysed using the Number Cruncher Statistical System version 11.

Results: There were 16 studies published between 1989 and 2018 that reported improvement in the overall survival (p=0.05) despite the reported significant heterogeneity across the studies (I^2=70%). Exploiting amino acid metabolic vulnerabilities was associated with a favourable prognostic outcome (p=0.05), while targeting glycolysis and nucleic acid synthesis had no significant clinical importance (p>0.05).

Conclusion: There is an urgent need to develop future therapies relying on the synergistic actions of nucleotide biosynthesis, glycolysis and amino acid metabolism.

Key Words: Metabolic vulnerabilities, Cancer, Chemotherapy, Cell metabolism, Metabolic enzymes.

Introduction

Aberrant metabolic activities in cancer cells have merited the attention of researchers predating the detection of tumour suppressors and oncogenes by approximately 50 years. The knowledge regarding cancer metabolism was initially inspired by Otto Warburg,[1] who reported a 10-fold increase in the catabolism of glucose carbon to lactate in tumour cells compared to normal cells even in the existence of oxygen. This metabolic alteration was supposedly attributable to mitochondrial defects that precluded their capacity of glucose carbon oxidation to carbon dioxide.[1] As such, 18F-deoxyglucose positron emission tomography (FDG-PET) has been utilised in cancer detection, showing an efficient clinical promise.[2]

Nonetheless, recent understanding of cancer biology has revealed contradictory observations. The aerobic glycolysis exhibited by tumour cells is not inherently related to mitochondrial dysfunctionality or impaired oxidative phosphorylation, but rather ascribed to a "reprogrammed" mitochondrial metabolism that leads to an increase in the macromolecular synthesis.[3] Indeed, reprogramming is a complicated process that can be mediated by mutagenesis or epigenetic modifications in tumour suppressor genes, such as Von Hippel–Lindau tumour suppressor (VHL), retinoblastoma (Rb), and tumour protein 53 (p53), or oncogenes, such as nuclear factor erythroid 2-related factor 2 (NRF2) and the P110α-encoding gene PIK3CA.[4] Furthermore, other cellular factors can influence cancer metabolism, including nutrient limitation, cellular interaction and oxygen availability.[4] Moreover, the ability of a given oncogene to change metabolism in a specific tissue but not another has raised the possibility of tissue-specific signalling involvement.[5]

The variation in metabolic dependencies of cancer cells created a considerable number of metabolic liabilities that could be targeted therapeutically. These therapies exploit vulnerable aspects critical for tumour growth and survival and, hence, could be clinically useful. However, metabolomic studies were primarily performed in cancer cell lines rather than the pathogenic tumours,[6] demonstrating the potential molecular mechanisms involved in metabolic reprogramming as well as altered signalling pathways. Culture-based experimental models may yield different outcomes when compared to the real oncogenic microenvironment. It is also worthy to note that the metabolic liabilities in some in vivo studies have not been previously reported in their counterparts conducted on culture cells.[5] Given that the novel therapeutic strategies against cancer are either in use clinically or being assessed in the preclinical and clinical settings, this systematic review was planned to provide an insight into the most recent knowledge about cancer metabolism and how the therapeutic targets could be approached, focussing on the efficacy and safety of them.

Methods

The systematic review and meta-analysis of studies that investigated a potential metabolic vulnerability to be exploited in any cancer was conducted at from Articles included had to be in English, published in a peer-reviewed journal and its full version was available. The systematic review relied on a literature search of the exploitable vulnerabilities based on updating and extending a previously-published review.[7] The identified clinically-targetable aspects of cancer metabolism included glycolysis, fatty acid metabolism, tricarboxylic acid cycle and mitochondrial metabolism, amino acid metabolism and nucleic acid synthesis. Cohort studies and randomised clinical trials (RCTs) were included in the systematic review.

For meta-analysis, the eligible studies were those which compared survival outcomes in an intervention group with a control group and reported the hazard ratios (HRs) as indicators of patients' survival following the administration of anticancer drugs. HR was used as a marker to analyse survival since this measurement is more preferably employed in meta-analyses over median survival times or survival rates.[8]

Articles excluded were experimental investigations performed on cell cultures, literature reviews, meta-analyses and case reports. Additionally, studies with sample sizes <20 were also excluded. There was no limit for the publication date in the search process.

The search process

The review was based on the guidelines of the Preferred Reporting Items for Systematic Review and Meta-analysis protocols (PRISMA-P).[9] As such, the patient (P), intervention (I), control (C), and outcome (O), PICO, framework was used to structure and develop the search strategy according to the Cochran's handbook of systematic reviews of interventions.[10]

Databases searched for eligible articles were PubMed, Clinicaltrials.gov, Embase, and Google Scholar. Search terms used were: cancer metabolism AND patients, cancer metabolism AND clinical trial, glycolysis AND cancer AND patient, glycolysis AND cancer AND patient, gluconeogenesis AND cancer AND patient, fatty acid metabolism AND cancer AND patient, TCA (tricarboxylic acid) cycle and mitochondrial metabolism AND cancer AND patient, amino acid metabolism AND cancer AND patient, and nucleic acid synthesis AND cancer AND patient. Screening of eligible studies was performed through their titles and abstracts until October 2018. The bibliographies of all pertinent articles were searched for available studies to be possibly included.

Data collection and extraction

Search results were uploaded onto Endnote software (Clarivate Analytics, Philadelphia, United States). Following the screening process, the abstracts and

full texts were analysed, and eligible articles were included. For the systematic review, retrieved data, including authors' names, year of publication, sample size, study design, metabolic target, mode of action, potential anticancer agent, stage of development, target cancer, and HR and/or median overall survival (OS) with a 95% confidence interval (CI) were extracted into a specifically-designed table. The mode of action of cancer therapy was either expressed as normalisation or depletion, where the former meant causing readjustment of the conversion rates of metabolites toward those occurring in normal metabolic pathways evidently in healthy cells, while the latter indicated inhibition of essential pathways critical for tumour cell growth.

Data extraction was performed by two independent researchers and failure to reach a consensus was resolved by either discussion or consulting a third reviewer. When available, the reported HR was collected and integrated for subsequent analysis.

Outcomes and test hypothesis

The primary outcome was reporting the median OS or HR of OS for an agent that targeted a metabolic vulnerability in cancer cells. Based on an extensive literature search, it was hypothesised that metabolic anticancer agents had significant effect on improving patients' survival.

Quality assessment

The quality assessment of the included studies was conducted using the Newcastle-Ottawa quality assessment scale[11] for cohort and case-control studies. It included a quality assessment of distinct criteria, including selection and comparability of the study groups as well as ascertainment of exposures or outcomes. In such scales, a specific star system is employed where both cohort and case-control studies are assigned a maximum of two stars for the comparability item, while other items can be awarded a maximum of one star for each, yielding a 0-9 range. Low-quality articles were deemed at score 0-3, moderate quality at 4-6 and high quality at 7-9. On the other hand, the Jadad

score[12] was used to assess the quality of Phase III RCTs according to randomisation and double-blinding. Such method relies on a score ranging between 0 (bad) and 5 (good), using a 7-item scale, where the last two items deduct a negative score. Phase I/II trials were not assessed for their quality due to lack of relevant scales, yet their results were considered in the qualitative research.

Statistical analysis

Meta-analysis was performed only on the comparative studies reporting HRs with 95% CIs as indications for the efficacy of targeting the metabolic vulnerabilities. When they were not available, HRs and CIs were calculated from the median survival times using the survival parameter conversion tool, which is integrated into the Number Cruncher Statistical System version 11 (NCSS 11) statistical software (NCSS, LLC, Utah, US). The pooled effect of relevant therapies on OS was calculated using the Review Manager (RevMan) 5.3 software (The Cochrane Collaboration, Oxford, United Kingdom). The Q test and the I-squared test were used to measure heterogeneity between studies, where a statistically-significant heterogeneity was deemed at $p<0.05$ or $I^2>50\%$. In the latter instance, the random effect model was applied.

Results

Initially, the search yielded 2,754 articles retrieved from the different databases with an additional five studies identified from the bibliographies of the screened articles. Of them, 36(1.3%) studies were assessed for inclusion, and 28(78%) of them were included in the systematic review, while 16(44.4%) were considered for the meta-analysis (Figure 1).

A summary was generated of the included studies that investigated the survival data of patients treated with drugs acting on the metabolic vulnerabilities These studies were published between 1989 and 2018 (Tables 1-3). Regarding study design, 5(18%) articles were retrospective cohort studies,[13-17] 8(28.5%) were

phase I/II trials,[18-25] and 15(53.5%)were randomised phase III trials. The authors in 11(39.3%) studies used the anticancer agent as a single therapy,[13-15, 23-30] while it was combined with radiotherapy in 6(21.4%) studies[17, 21, 22, 31-33] and with other chemotherapeutic drugs in the remaining 11(39.3%) studies.

For quality assessment, the scores of nonrandomised studies ranged 6-7, indicating a moderate to high quality. The inability to demonstrate the lack of outcomes at the start of the study was inconsistently reported in cohort studies, while the lack of reporting non-response rates was the deficient item in case-control studies (Table). Most of the RCTs scored 3 since only one study employed a double-blinding methodology.[31] Lower scores were attributable to either the lack of reporting the method of randomisation[32, 34] or inappropriate methods of randomisation[35] (Table).

In general, in the five clinically-targetable aspects of cancer metabolism, there was a significant heterogeneity (P for heterogeneity [P_h]<0.001; I^2=70%) and the overall effect tended to improve patients' survival (HR: 0.87; 95% CI: 0.76-1.00; p=0.05; Figure 2).

Glycolysis

Disruption of glycolysis could be performed via several agents. Lonidamine (LND) is an established inhibitor of the hexokinase (HK) II enzyme, which is involved in the conversion of glucose to glucose-6-phosphate (G6P) as the first step in glycolysis following glucose entry, thereby preserving energy consumption within tumour cells.[36] LND was used in combination with other anticancer therapies in all studies, showing OS improvement in breast cancer[20] and solid malignant tumours.[18, 19] However, targeting HKII by LND in RCTs showed no significant prolongation of the OS compared to the methotrexate-doxorubicin-cyclophosphamide–lomustine (CCNU) therapy (MACC)[37] or radiation therapies.[31, 32] Furthermore, subsequent phase clinical trials on benign prostatic hyperplasia were discontinued as a result of the lack of adequate therapeutic efficacy or the development of severe side effects (Clinicaltrials.gov

identifiers: NCT00435448, NCT00237536). Given the difference in the outcomes, the present meta-analysis revealed no significant therapeutic effect of targeting glycolysis using LND and the included studies showed a significant heterogeneity (HR: 1.00; 95% CI: 0.73-1.39; p=0.98; P_h: 0.06; I^2=65%; Figure 3A).

Also, 2-deoxyglucose (2-DG) is another glucose analogue that is phosphorylated by HKII. The phosphorylated form of 2-DG accumulates in the cells as it is not affected by G6P, thereby glycolysis is halted.[38] Oral 2-DG administration was well-tolerated in patients with brain tumours at doses up to 250mg/kg bodyweight (BW) when combined with radiation therapies,[21, 22] while the optimum dose of 2-DG in advanced solid tumours was determined at 45mg/kg since higher doses caused asymptomatic prolongation of corrected QT interval (QTc).[39] However, the efficacy of such an agent as a therapeutic approach was questionable.[40]

For potential cancerous vulnerabilities under investigation, a Phase I dose-escalating trial showed a lack of dose-limiting toxicity of 6-phosphofructo-2-kinase-158 (PFK-158), which is a potent inhibitor of PFK/fructose 2,6-bisphosphatase (PFKFB3), rendering this agent as the first PFKFB3 inhibitor in human (Clinicaltrials.gov identifier: NCT02044861). TLN-232 is another essential regulatory agent of glycolysis, via inhibition of pyruvate kinase M2, that has been investigated in a Phase II clinical trial in patients having refractory metastatic melanoma (Clinicaltrials.gov identifier: NCT00735332). However, the study was terminated due to the termination of the manufacturer's license.

Fatty acid metabolism

For drugs affecting cancer metabolism, only statins have been successfully identified as reducing the risk of cancer,[41] possibly by inhibiting hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase that leads to a potent growth inhibition effect on cancer cells.[42] However, large-sized retrospective cohort studies have shown a significant impact on OS in patients

with ovarian and breast cancer, with a more prominent role of simvastatin when compared to other drugs.[13, 14]

Other aspects of lipid metabolic weaknesses of cancer cells are still being investigated in clinical trials. Fatty acid synthase (FAS) is a targetable enzyme involved in the production of membrane phospholipids essential for cancer cell membranes.[43] Epigallocatechin gallate (EGCG) is a FAS inhibitor that showed high tolerability, causing a sustained reduction of the absolute lymphocytic count in 69% of patients with Rai stage I to II chronic lymphocytic leukaemia (n=42).[44] TVB-2640 is another FAS inhibitor which is currently under investigation on three different types of cancer, while only one clinical trial was being held on a novel choline kinase alpha (Chk-α) inhibitor named traslational cancer drug (TCD)-717.[45-47]

TCAcycle and mitochondrial metabolism

Given that mitochondrial metabolism in cancer cells is not only dependent on glucose-derived pyruvate but also on fatty acids, lactate and amino acids, such as glutamine, to supply carbon sources for the TCA cycle,[48] multiple metabolic weaknesses could be targeted in these pathways. Dichloroacetate (DCA) has completed a Phase I trial in 8 patients with recurrent malignant brain tumours and was not associated with dose-limiting toxicities[23], yet further investigations were not performed. Interestingly, a new era of acute myeloid leukaemia (AML) treatment has emerged with the introduction of two recently-approved agents that induce glutaminolysis in AML patients. Ivosidenib (AG-120) has been approved at a dose of 500mg daily, showing an overall response in 41.6% patients (95%CI: 32.9-50.8) with a low frequency of grade 3 or higher adverse effects in patients with mutant isocitrate dehydrogenase (IDH)-1 mutant AML.[24] Targeting the mutant IDH-2 enzyme by the potent inhibitor enasidenib (AG-221) was also evaluated in a Phase I escalation trial[25] with an overall response rate of 40.3% and a significant hematologic response.

Metformin is another antidiabetes agent that could be assessed for its metabolically-active role in cancer. Nonetheless, observational studies[49] and large-base prospective investigations[50] have revealed that the impact of metformin was only limited to reducing the risk of cancer in patients with type 2 diabetes, or reducing the overall mortality in diabetic metformin-receiving patients compared to non-diabetic individuals (HR: 0.85; 95% CI: 0.78–0.93).[15]

For the ongoing trials, the therapeutic effects of CB-839 are being investigated in multiple types of cancer after inducing reversible side effects (elevation of transaminases) at doses ranging between 100 and 1000mg ter in die (TID), or three times a day, in patients with relapsed/refractory leukaemia.[51] Lactate utilisation by tumour cells might also be regarded as a therapeutically-liable vulnerability that is being approached clinically by the monocarboxylate transporter inhibitor AZD3965 (Clinicaltrials.gov identifier: NCT01791595).

Amino acid metabolism

L-asparaginase has been approved in the standard regimen of acute lymphoblastic leukaemia (ALL) and related lymphoma as it depletes asparagine necessary for cancer cells. The effects of such therapy were likely to be more favourable in children with ALL rather than the adolescent and adult populations, where adverse side effects, such as pancreatitis, hypersensitivity reaction, and thrombosis, have been frequently reported.[16, 34, 52] Arginine is another amino acid that could be available for cancer cells and, hence, therapies that deplete exogenous arginine may be effective, particularly in argininosuccinate synthetase-1 (ASS-1)-deficient cancers.[53] The use of pegylated arginine deiminase (ADI-PEG20) has yielded a remarkable improvement in progress-free survival (PFS) in patients with ASS-1-deficient pleural mesothelioma (HR: 0.56; 95% CI: 0.33-0.96; p=0.02).[54]

Another possible mechanism that could be exploited in cancer metabolism is the inhibition by difluoromethylornithine (DFMO) of ornithine decarboxylase,

286 which leads to increased polyamine levels and tumorigenesis,. Although
287 Saulnier Sholler et al.[55] have shown that children with polyamine-dependent
288 relapsed neuroblastoma are susceptible to DFMO with remarkable safety and
289 tolerability, two RCTs have revealed no survival benefits of adding DFMO with
290 procarbazine-lomustine–vincristine (PCV) therapy in patients with glioblastoma
291 and astrocytoma.[56, 57] Overall, the included studies that targeted amino acid
292 metabolism had the most favourable effect estimates on OS as indicated by a
293 pooled HR of 0.67 (95% CI: 0.45–1.00; p=0.05). However, these results should
294 be cautiously interpreted given the significant inconsistency between the
295 included studies (P_h<0.01; I^2=84%; Figure 3B).

296 **Nucleic acid synthesis**

297 Gemcitabine is an antimetabolite nucleoside analogue that inhibits
298 deoxyribonucleic acid (DNA) polymerase and, thus, can be used to treat cancer.
299 The survival outcomes of this agent were conflicting. Four RCTs investigated
300 the use of a single gemcitabine therapy, showing an additional survival benefit
301 rather than observation following pancreatic cancer resection[27, 28] but not
302 following bile duct cancer resection.[26] Furthermore, patients with pancreatic
303 malignancies who received a folinic acid-fluorouracil-irinotecan-oxaliplatin (FOLFIRINOX)
304 therapy showed better survival outcomes when compared to those who received
305 gemcitabine[29].

306 Similarly, the impact of 5-fluorouracil (5-FU) on exploiting cancer metabolic
307 vulnerabilities through the inhibition of DNA synthesis was inconsistent in the
308 RCTs. While 5-FU administration yielded a significant increase in the five-year
309 survival rate after pancreatic cancer resection when compared to a combination
310 of 5-FU and radiotherapy (21% versus 8%, respectively; p=0.009),[30] there was
311 no significant beneficial effects of adjuvant chemotherapies containing 5-FU,
312 alpha interferon and interleukin-2 (IL-2) or 5-FU and capecitabine following
313 nephrectomy for renal cell carcinoma mesorectal excision for rectal cancer,
314 respectively.[33, 58] As such, drugs that target nucleic acid synthesis showed no

significant effect on patients' survival as an indication of targeting the metabolic cancer weaknesses (HR: 0.93; 95% CI: 0.78-1.12; p=0.93; Figure 3C).

Discussion

Several metabolic vulnerabilities have been successfully exploited in cancer cells, showing varied efficacy degrees, but their use may be limited by their toxicities rather than by their cancer cell-killing capabilities. The present study reviewed the possible clinically-exploited weaknesses and investigated the effects of targeted therapies through their HRs to compare their efficacy, tolerability and patients' prognosis. The most commonly investigated targetable changes were glycolysis, glutaminolysis and nucleic acid synthesis.

Our results emphasised the significance of targeting amino acid metabolism. Cancer cells show an increased demand for distinct amino acids that might be considered a "metabolic addiction". Such characteristics involve increased nitrogen requirements for biosynthesis, increased amino acid consumption, and elevation of their relevant transporters, increased dependence on exogenous non-essential amino acids that exceeds the capacity of intracellular supply and altering the levels of amino acid-specific catalytic enzymes.[59] Bu et al.[17] found that a combination of L-asparaginase and radiotherapy was associated with a remarkably increased OS compared to a cyclophosphamide-hydroxydaunorubicin-vincristine–prednisone (CHOP) regimen in patients with extranodal natural killer cell/T-cell lymphoma (77 versus 34 months, respectively; p<0.001).

The present systematic review showed that targeting glucose uptake at the early steps of glycolysis was not efficacious. This could be explained by the fact that glucose uptake is also enhanced in non-cancerous tissue, including the brain, as shown by FDG-PET scans,[60] indicating that glucose uptake is not a unique property of cancer cells. As such, direct targeting of such vulnerability may be

limited by the synergistic effects that may be exerted by 2-DG therapies on normal cells. However, efforts in the preclinical studies are still ongoing to exploit other aspects of glycolysis, such as the use of glucose transporter (GLUT) inhibitors (WZB117 and Fasentin) for clinical use.[61] Moreover, Cervantes-Madrid et al.[62] have reasonably suggested the renewal of the LND-concerned studies, particularly when it is combined with 6-diazo-5-oxo-L-norleucine (DON) to target glycolysis and glutaminolysis, respectively. Importantly, targeting the enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) by 3-bromopyruvate (3-BrPA) is possible, providing an energy-depleting approach specific to cancer cells.

The clinical success of targeting nucleic acid synthesis was variable in the included RCTs in the present study. Although the increased metabolic demand of cancer cells to DNA replication and nucleotide synthesis is crucial, the concomitant destruction of other highly proliferative normal cells in the body, including intestinal crypts, hair follicles and bone marrow, may limit the overall effects of antimetabolites that may be associated with dose-limiting toxicities, such as myeloid suppression and gastrointestinal toxicities.[63]

Gemcitabine was efficient as a single therapy although its efficacy was inferior to the FOLFIRINOX therapy. Its cytotoxic effect showed a potent synergism with cisplatin and such combination may be regarded as the first-line treatment of nasopharyngeal carcinoma.[64] Moreover, the adjuvant combination of capecitabine and gemcitabine was superior to gemcitabine alone as a post-surgical therapy for pancreatic ductal carcinoma.[65] However, intrinsic or acquired gemcitabine resistance has been reported in pancreatic cancer elsewhere,[66] and recent preclinical evidence has revealed that such resistant cells could be sensitised by disruption of the glutamine pathways via an adjuvant DON therapy.[67] The synergistic action of antimetabolite drugs and glutaminolytic drugs could be explained by the interference of nucleotide biosynthesis with other metabolic pathways, where glucose requirements for

ribose synthesis are derived from the pentose-phosphate pathway while aspartate and glutamine provide the required nitrogen atoms for nucleotide bases. Indeed, these essential synergistic actions would potentially widen the therapeutic solutions.

Considering mitochondrial metabolism, the present review highlighted the clinical importance of two novel agents against relapsed/refractory AML that target mutant IDH isoforms without inducing significant bone marrow aplasia. More specifically, the prolonged OS of enasidenib in elderly patients with advanced myeloid malignancies was remarkable and comparable with shorter survival outcomes in a randomised Phase 1/2 study.[68] From another perspective, new horizons in cancer metabolism could be elucidated by targeting fatty acid synthesis since the current confirmed evidence is scarce and only limited to statins. For example, Penet et al.[69] recommended evaluating TCD-717 as a Chk-α inhibitor in the treatment of pancreatic cancer cells as they usually express impaired choline metabolism. The ongoing clinical trials could provide a better insight into the prospected clinical effectiveness.

Targeting the metabolic vulnerabilities would possibly open new therapeutic windows, particularly in light of the need to reduce enzyme-mediated resistance to chemotherapeutic agents, including paclitaxel resistance in breast cancer,[70] hypoxia-induced resistance in solid tumours,[71] and cisplatin resistance in gastric cancer.[72]

Observational and retrospective studies were excluded from our meta-analysis to yield more reliable outcomes and to avoid significant time-related biases. However, this study may have some limitations. First, the available clinical data is still insufficient to conclude a robust approach for further investigations in cancer metabolism despite the significance of amino acid metabolism. Second, determining HR as a primary outcome in the present study might lead to reducing the number of included studies, and thereby some articles may have escaped inclusion. Third, the significant inconsistencies among the included

studies in our meta-analysis, possibly due to variation in the combined therapies and control groups, might render a difficult interpretation.

Conclusion

Current evidence has shown that metabolically-active drugs tend to improve OS of patients with solid tumours with a relatively greater effect via exploiting changes in amino acid metabolism. L-asparaginase and ADI-PEG20 are the most acceptable anticancer agents that could be used for the treatment of paediatric and adult ALL and ASS1-negative cancers, respectively. However, combining chemotherapeutic regimens that target the dysregulated metabolic aspects seems to induce better outcomes in terms of efficacy and safety. Given the interfering pathways in nucleotide biosynthesis with glycolysis, folate metabolism and amino acid metabolism, future synergistic therapies should be developed, investigated in preclinical models and employed in the clinical setting as appropriate. Finally, the development of novel cancer-targeted therapies based on tumour metabolism is mainly dependent on conducting feasible in vivo studies, utilising advanced imaging techniques, and testing potent, selective inhibitors that can be safely introduced to the patients.

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: None.

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Table 1: The established metabolic vulnerabilities (Glycolysis) and their targeting agents.

Agent	Target cancer	Study Design	Single/Combined therapy	Reference Group	Sample size*	Outcomes	HR/MOS (95%CI)	Reference
Glycolysis								
LND	Advanced NSCLC	Clinical trial	+MACC regimen	None	46	Improved overall survival with tolerable side effects	MOS: 42 weeks (20-52)	Buccheri et al.[18]
	Advanced ovarian cancer	Clinical trial	+Paclitaxel, cisplatin	None	35	Improved overall survival with tolerable side effects	MOS: 46.5 months (32.4-60.00)	De Lena et al.[19]
	Advanced NSCLC	RCT	+MACC regimen	MACC alone	75	No improvement in the overall survival with significant side effects	HR: 0.89 (0.59-1)	Buccheri et al.[37]
	Breast cancer	Clinical trial	+EPI and ciplastin	None	30	Improved overall survival with tolerable side effects	MOS: 14+ months	Gebbia et al.[20]
	Advanced NSCLC	RCT	+radiation therapy	Placebo +radiation	158	No improvement in the overall survival with significant side effects	HR: 0.83 (0.63-0.97)	Scarantino et al.[31]

	Brain metastatic cancer	RCT	+WBR	WBR	31	No significant differences	HR: 1.37 (0.99-1.90)	DeAngelis et al.[32]
2-DG	Cerebral glioma	Clinical trial	+large-fraction radiation therapy	None	20	Well-tolerated with no significant side effects.	MOS: 30 months	Mohanti et al.[21]
	Glioblastoma multiforme	Clinical trial	radiation therapy	None	10	Well-tolerated with no acute toxicity	MOS: 24 months	Singh et al. [22]

Table 2: The established metabolic vulnerabilities (Fatty acid metabolism, TCA cycle and mitochondrial metabolism, Amino acid metabolism and their targeting agents).

Fatty acid metabolism								
Statins	Ovarian cancer	Cohort	Single	Non-statin use	5416	Improved survival	HR: 0.81, (0.72–0.90)	Couttenier et al.[13]
	Breast Cancer	Cohort	Single	Non-statin use	4,151	Improved survival	HR: 0.80, (0.65–0.98)	Murtola et al.[14]
TCA cycle and mitochondrial metabolism								
DCA	Recurrent brain tumours	Clinical trial	Single	None	15	Feasible and well-tolerated	MOS: 140 days (range 75–146)	Dunbar et al.[23]
AG-120	R/R IDH1-Mutated AML	Clinical trial	Single	None	179	Good response with few adverse events	MOS: 14.5 months (13.9–15.3)	DiNardo et al.[24]
AG-221	R/R IDH2-Mutated AML	Clinical trial	Single	None	214	Well-tolerated with promising hematologic responses	MOS: 9.30 months (8.20–10.9)	Stein et al.[25]

Metformin	Various	Cohort	Single	Subjects without diabetes	112,408	Significant improved survival	HR: 0.85, (0.78-0.93)	Currie et al.[15]
Amino acid metabolism								
L-asparaginase	PTCL	Retrospective	+multidrug chemotherapy	chemotherapy	42	Acceptable short-term therapeutic effects with reversible side effects	HR: 0.52, (0.30-1.2)	Yao et al.[16]
	NKTCL	Retrospective	+ radiotherapy	CHOP regimen	112	Well-tolerated and highly effective	HR: 0.44, (0.31-0.62)	Bu et al.[17]
	ALL	RCT	+ AdVP	AdVP only	98	No significant differences in the therapeutic and safety outcomes	13.5 months vs 17 months for the control	Nagura et al.[34]
ADI-PEG20	Pleural Mesothelioma	RCT	+ BSC	BSC only	44	Significant improvement in overall survival and manageable side effects	HR: 0.68, (0.39-1.16)	Szlosarek et al.[54]
DFMO	Glioblastoma multiforme	RCT	+ PCV	PCV only	134	No additional benefits	HR: 1.00, (0.91-	Levin et al.[56]

							1.11)	
	Anaplastic astrocytoma	RCT	+ PCV	PCV only	33	No additional benefits	HR: 0.80, (0.50-1.1)	Levin et al.[57]

Table 3: The established metabolic vulnerabilities (Nucleic acid synthesis) and their targeting agents.

Nucleic acid synthesis								
Gemcitabine	Resected bile duct cancer	RCT	Single	Surgery only	117	No additional benefits	HR: 1.01, (0.7-1.45)	Ebata et al.[26]
	Resected pancreatic cancer	RCT	Single	Surgery only	179	Significant increase in OS	HR: 0.76, (0.61-0.95)	Oettle et al.[27]
	Resected pancreatic cancer	RCT	Single	Surgery only	58	Significant increase in OS	HR: 0.77, (0.51-1.14)	Ueno et al.[28]
	Metastatic pancreatic cancer	RCT	Single	FOLFIRINOX therapy	171	Gemcitabine is less effective	HR: 1.75, (0.87-1.96)	Conroy et al.[29]
5-FU	Colon Cancer	RCT	+ folinic acid	Capecitabine and	942	Capecitabine and Oxaliplatin therapy was superior	HR: 1.20, (1.01-1.43)	Schmoll et al.[35]

				Oxaliplatin				
	Resected pancreatic cancer	RCT	Single	5-FU with radiotherapy	220	Significant increase in OS	HR: 0.70, (0.49-1.01)	Neoptolemos et al.[30]
	Excised rectal cancer	RCT	+ radiotherapy	Surgery only	216	No additional benefits	HR: 1.07, (0.72-1.61)	Breugom et al.[33]
	Recurrent renal cell carcinoma	RCT	+ alpha-interferon and interleukin-2	Observation	154	Significant toxicity with no additional benefits	HR: 0.84, (0.63-1.12)	Aitchison et al.[58]

** Sample size of the intervention group; 2-DG: 2-deoxyglucose; 5-FU: Fluorouracil; AdVP: doxorubicin, vincristine and prednisolone; ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; BSC: best supportive care; DCA: dichloroacetate; DFMO: difluoromethylornithine; HR: hazard ratio; IDH: Isocitrate dehydrogenase; LND: Lonidamine; MACC: methotrexate, doxorubicin, cyclophosphamide, and CCNU; MOS: Median overall survival; NKTCL: natural killer cell T cell lymphomas; NSCLC: non-small cell lung cancer; PCV: Procarbazine, lomustine and vincristine; PTCL: Peripheral T-cell lymphoma; R/R: relapsed/refractory; RCT: randomised clinical trial; TCA: tricarboxylic acid cycle; WBR: Whole brain radiation.*

Table 4: Results of quality assessment of the included cohort and case-control studies in the systematic review based on the Newcastle-Ottawa Scale.

	Selection				**Comparability**	**Exposure/Outcome**			**Total score**
Cohort Studies	Representativeness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	demonstrate the lack of outcome at the start of the study	Comparability of cohorts	Assessment of outcome	follow-up long enough	Adequacy of follow up	
Couttenier et al.[13]	✵	✵	✵		✵	✵	✵	✵	7
Murtola et al.[14]	✵	✵	✵		✵	✵	✵	✵	7
Currie[15]	✵	✵			✵	✵	✵	✵	6
Case-control studies	Adequacy of case definition	Representativeness of the cases	Selection of Controls	Definition of Controls	Comparability of cohorts	Ascertainment of exposure	ascertainment for cases and controls	Non-Response rate	
Yao et al.[16]	✵	✵		✵	✵✵	✵	✵		7
Bu et al.[17]	✵	✵		✵	✵	✵	✵		6

Table 5: Quality assessment scores of the randomised clinical trials included in the systematic review based on the Jadad Score.

Study	Randomisation mentioned	Method of randomisation described	Double blinding mentioned	Method of double blinding described	Description of withdrawals and dropouts	Inappropriate method of randomisation	Inappropriate method of double blinding	Total score
Buccheri et al.[37]	1	1	0	0	1	0	0	**3**
Scarantino et al.[31]	1	1	1	0	1	0	0	**4**
DeAngelis et al., [32]	1	0	0	0	1	0	0	**2**
Nagura et al.[34]	1	0	0	0	1	0	0	**2**
Szlosarek et al.[54]	1	1	0	0	1	0	0	**3**
Levin et al.[56]	1	1	0	0	1	0	0	**3**
Levin et al.[57]	1	1	0	0	1	0	0	**3**
Ebata et al.[26]	1	1	0	0	1	0	0	**3**
Oettle et al.[27]	1	1	0	0	1	0	0	**3**

Ueno et al.[28]	1	1	0	0	1	0	0	**3**
Conroy et al.[29]	1	1	0	0	1	0	0	**3**
Schmoll et al.[35]	1	1	0	0	1	-1	0	**2**
Neoptolemos et al.[30]	1	1	0	0	1	0	0	**3**
Breugom et al.[33]	1	1	0	0	1	0	0	**3**
Aitchison et al.[58]	1	1	0	0	1	0	0	**3**

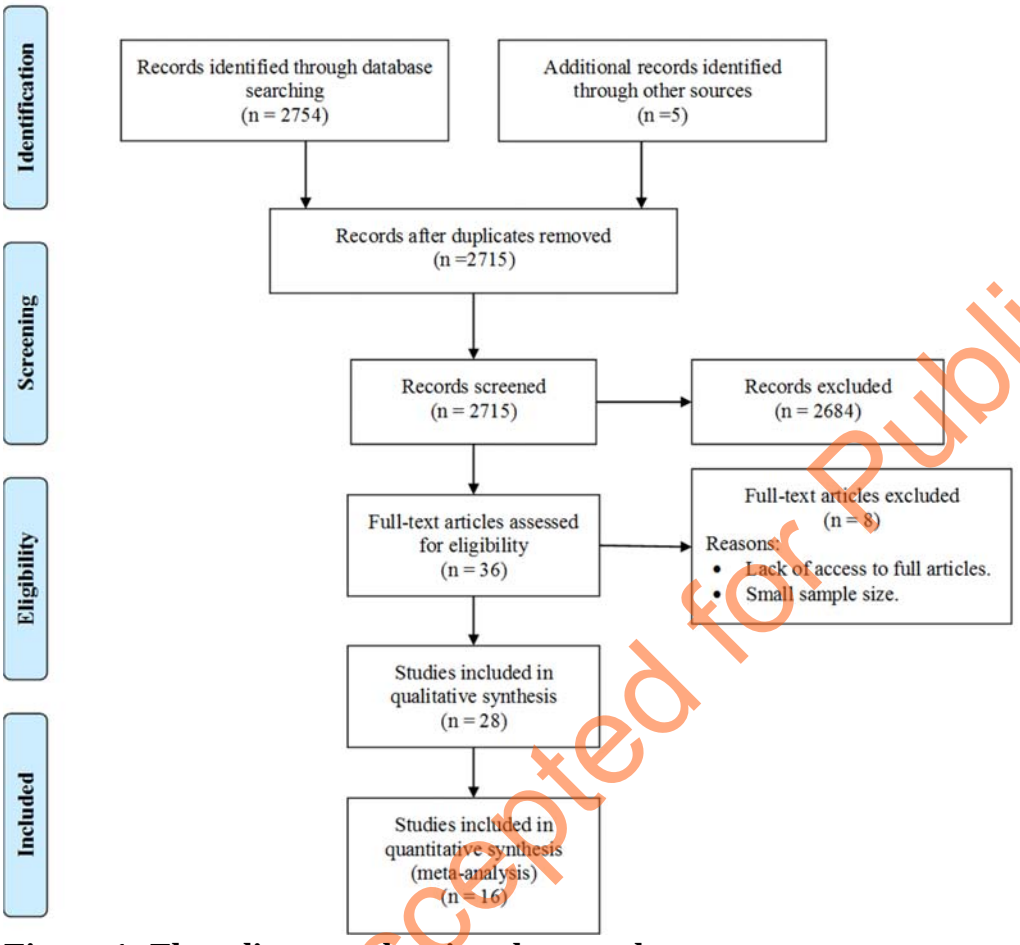

Figure 1: Flow diagram showing the search process.

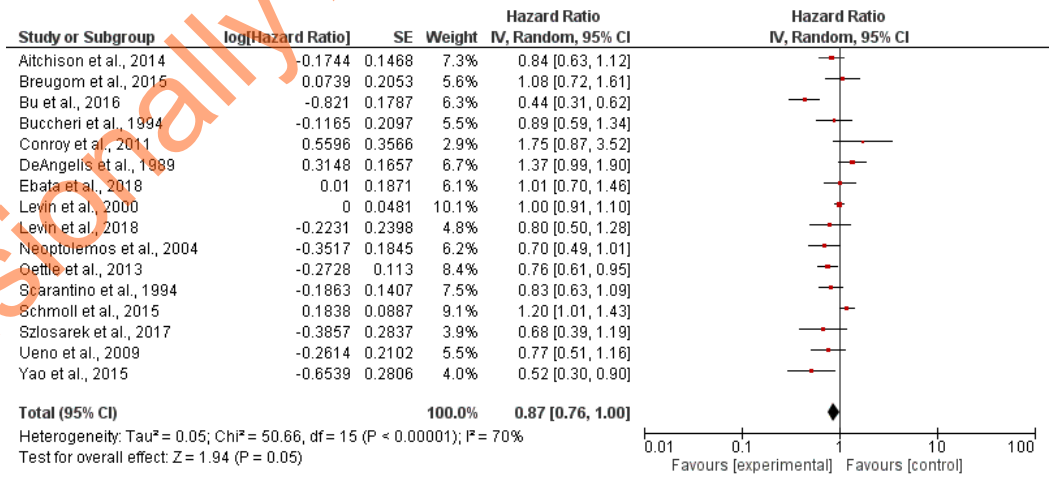

Figure 2: Forest plot of the overall effect of targeting cancer metabolic vulnerabilities.

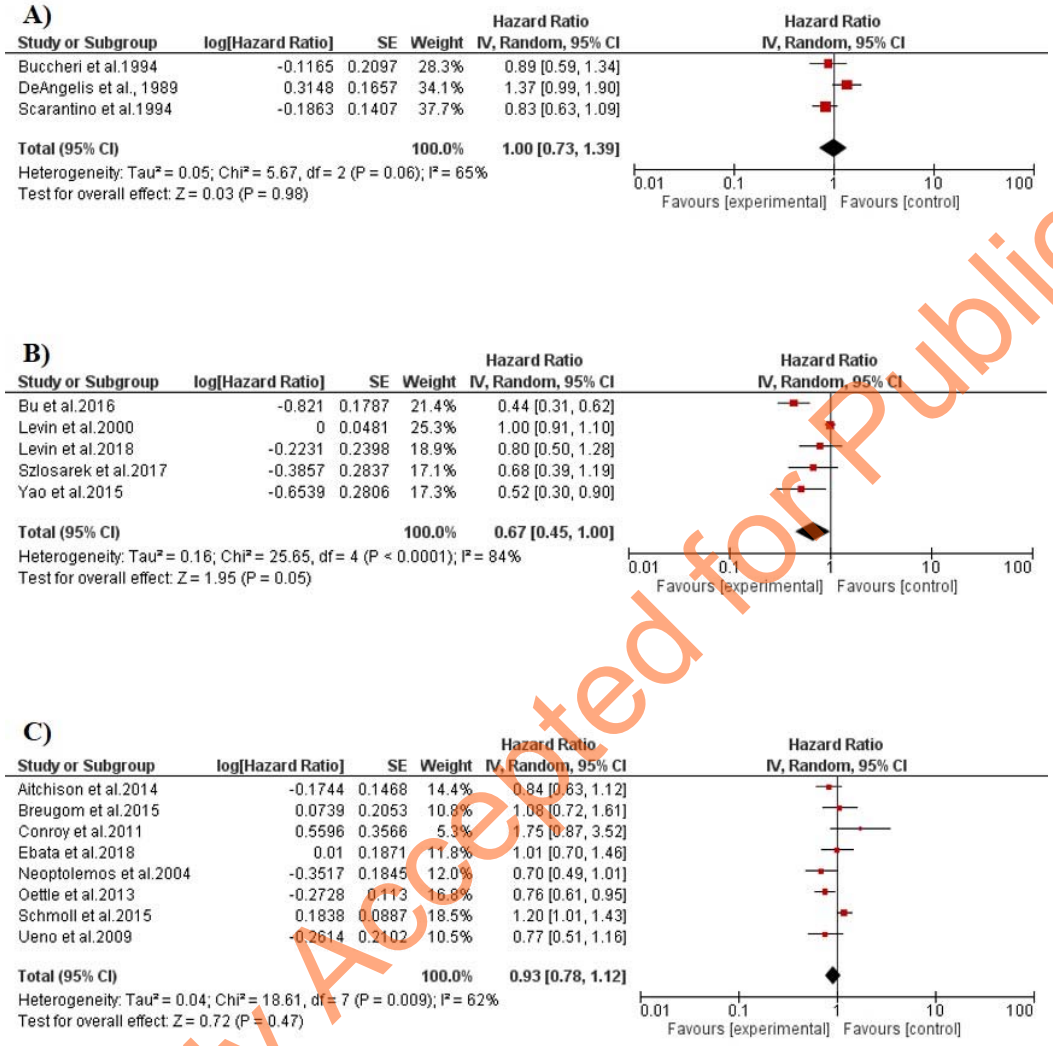

Figure 3: Forest plot of subgroup analysis of the effect of targeting the following vulnerabilities in cancer metabolism A) glycolysis B) amino acid metabolism C) nucleic acid synthesis.