

# Arginine Deprivation in SCLC: Mechanisms and Perspectives for Therapy

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**Abstract:** Arginine deprivation has gained increasing traction as a novel and safe antimetabolite strategy for the treatment of several hard-to-treat cancers characterised by a critical dependency on arginine. Small cell lung cancer (SCLC) displays marked arginine auxotrophy due to inactivation of the rate-limiting enzyme argininosuccinate synthetase 1 (ASS1), and as a consequence may be targeted with pegylated arginine deiminase or ADI-PEG20 (pegargiminase) and human recombinant pegylated arginases (rhArgPEG, BCT-100 and pegzilarginase). Although preclinical studies reveal that ASS1-deficient SCLC cell lines are highly sensitive to arginine-degrading enzymes, there is a clear disconnect with the clinic with minimal activity seen to date that may be due in part to patient selection. Recent studies have explored resistance mechanisms to arginine depletion focusing on tumor adaptation, such as ASS1 re-expression and autophagy, stromal cell inputs including macrophage infiltration, and tumor heterogeneity. Here, we explore how arginine deprivation may be combined strategically with novel agents to improve SCLC management by modulating resistance and increasing the efficacy of existing agents. Moreover, recent work has identified an intriguing role for targeting arginine in combination with PD-1/PD-L1 immune checkpoint inhibitors and clinical trials are in progress. Thus, future studies of arginine-depleting agents with chemoimmunotherapy, the current standard of care for SCLC, may lead to enhanced disease control and much needed improvements in long-term survival for patients.

**Keywords:** small cell lung cancer, SCLC, arginine, argininosuccinate synthetase 1, ASS1, arginine deprivation, tumor microenvironment, immunotherapy

## Introduction

Small cell lung cancer (SCLC) is a notoriously lethal subtype of smoking-related thoracic cancer, accounting for 10–15% of global lung cancer incidence with a 5-year survival rate of less than 7%.<sup>1</sup> Treatments for SCLC have advanced slowly in the last few decades with much scope for improvement. Harnessing fundamental and novel biological insights will be critical to transforming the SCLC therapeutic landscape.

Derived from neuroendocrine precursor cells, SCLC is characterised by rapid tumor growth, genomic instability and early metastatic spread underlying the poor survival outcomes.<sup>2,3</sup> On a molecular level, in addition to universal TP53 and RB1 inactivation, SCLC is classified into four distinct subtypes according to the expression of ASCL1 (SCLC-A), NEUROD1 (SCLC-N), POU2F3 (SCLC-P) or YAP1 (SCLC-Y).<sup>4,5</sup> This molecular reappraisal of SCLC offers the possibility of novel personalised therapies. Inducing arginine deprivation is a promising therapeutic strategy for multiple cancer types including SCLC that are deficient in the arginine biosynthetic enzyme argininosuccinate synthetase 1 (ASS1). In the current review, we provide an update on arginine deprivation as a specific antimetabolite therapy focusing on SCLC both as monotherapy and in combination with selected therapies based on emerging preclinical and clinical data.

## Critical Use of Arginine in Healthy and Cancerous Cells

### Background

Arginine (2-amino-5-guanidinovaleric acid) is a semi-essential amino acid in mammalian cells.<sup>6</sup> As one of the 20 most common amino acids in humans, it is a highly versatile molecule serving as the precursor of key molecules such as urea,

nitric oxide, proline, polyamines or proteins.<sup>7</sup> Additionally, arginine can trigger secretion of molecules such as growth hormones, insulin or glucagon.<sup>8</sup> This metabolite is a core component of the urea cycle, the only known pathway in humans capable of recycling and eliminating the nitrogen-containing molecule ammonia.<sup>9,10</sup>

Arginine biosynthesis takes place within the urea cycle from the conversion of ornithine to citrulline in the mitochondria catalysed by the ornithine transcarbamylase (OTC) enzyme.<sup>11</sup> Citrulline can also be sourced from glutamine through a series of sequential intestinal enzymatic reactions which release the metabolite to the blood circulation.<sup>12</sup> Citrulline and aspartate undergo condensation to argininosuccinate by the ATP-dependent enzyme ASS1, the rate-limiting enzyme of the urea cycle. The last step is the conversion of argininosuccinate into arginine and fumarate, a member of the TCA cycle, by argininosuccinate lyase (ASL).<sup>8</sup> Arginine biosynthesis accounts for only 10–15% of whole-body arginine production, implying that cells rely primarily on the transport of extracellular arginine and therefore the cell microenvironment to maintain arginine levels.<sup>13</sup>

## ASS1 Modulation in Cancer

ASS1 inactivation confers arginine auxotrophy in cancer cells and thereby susceptibility to arginine-depleting agents.<sup>14</sup> Loss of ASS1 and sensitivity to arginine deprivation has been observed in numerous cancers including melanoma, hepatocellular carcinoma (HCC), malignant pleural mesothelioma, glioblastoma, sarcomas and haematological cancers.<sup>15–19</sup> Other malignancies, such as ovarian and colorectal cancer express high levels of ASS1 compared to corresponding normal tissues.<sup>20</sup> In SCLC, Kelly et al identified that 45% of tumor samples and 50% of cell lines were ASS1 negative highlighting the importance of arginine biosynthesis downregulation for this malignancy.<sup>21</sup> The mechanisms leading to altered ASS1 expression differ. Transcription factors such as c-Myc and HIF-1 $\alpha$  interacting with an E-box located at the ASS1 gene promoter are known to modulate the expression of ASS.<sup>22</sup> ASS1 expression is also under epigenetic regulation with promoter methylation first being identified in mesothelioma cell lines and primary tumors as a driver of ASS1 deficiency.<sup>16,23</sup> Moreover, ADI-PEG20 treatment induced ASS1 re-expression in an MPM cell line linked to decreased methylation of the ASS1 promoter.<sup>24</sup> Although the exact mechanism of ASS1 expression in SCLC is yet to be fully understood, Chalishazar et al showed that MYC directly affects the arginine dependency of SCLC cell lines.<sup>25</sup> As such, MYC-driven cells were more sensitive to arginine deprivation, the reverse of that described for melanoma and highlighting cell-of-origin as a key determinant of the arginine metabolome.<sup>8,26</sup>

Several studies have highlighted the functional and metabolic changes as a consequence of ASS1 dysregulation in cancer (Table 1). To summarise, ASS1 downregulation correlates frequently with more aggressive and chemoresistant cancer phenotypes including increased proliferation, migration, invasion, and metabolic changes affecting glycolysis and the TCA cycle.<sup>27</sup> Low levels of ASS1 were associated with worse overall survival for several cancer types including ovarian cancer, mesothelioma, non-small cell lung cancer, bladder cancer and osteosarcoma.<sup>28–32</sup> On the other hand, in gastric cancer, ASS1 expression has been linked with tumorigenesis, chemoresistance and poor outcomes.<sup>33</sup>

## Targeting Arginine for Cancer Treatment

A deeper understanding of the role of arginine and ASS1 in tumorigenesis has accelerated the exploitation of arginine auxotrophy for personalised anticancer therapy. Several enzyme therapeutics have been developed to have a synthetically lethal effect in arginine-dependent and urea cycle-dysregulated tumor cells.<sup>34</sup>

### Arginase (ARG)

ARG is an enzyme that catalyses the conversion of arginine into ornithine and urea. Recombinant human ARG1 has been conjugated with polyethylene glycol of 5kDa to increase its half-life creating the clinically usable molecule: rhArg-PEG (BCT-100). Xu et al evaluated the potency of rhArg-PEG (BCT-100) on several SCLC cell lines, revealing apoptosis induced by oxidative stress, G1 cell cycle arrest, and reduced arginine concentrations correlating with low levels of ASS1 and/or OTC.<sup>35</sup> rhArg-PEG (BCT-100) has been studied in several phase I and II clinical trials in patients with HCC, melanoma, prostate adenocarcinoma or leukaemia; however, no data are available specifically in SCLC.<sup>36–39</sup> Another rhArgPEG that is cobalt-substituted (Co-Arg1-PEG or pegzilarginase) also displayed robust activity in 8/12 patient-derived xenograft (PDX) models of SCLC and the related Merkel cell cancer, an aggressive skin neuroendocrine cancer.<sup>40</sup>

**Table I** Functional and Metabolic Effects of ASS1 Modulation in Cancer

Cancer Type	Functional/Metabolic Effect	Year	Study
Ovarian	<ul style="list-style-type: none"> <li>Downregulation of ASS1 was associated with the development of platinum-based treatment resistance and significantly reduced overall survival</li> </ul>	2009	Nicholson et al <sup>28</sup>
Osteosarcoma	<ul style="list-style-type: none"> <li>ASS1 deficiency increases tumorigenesis</li> <li>Low ASS1 correlated with the development of pulmonary metastasis</li> </ul>	2010	Kobayashi et al <sup>32</sup>
Bladder	<ul style="list-style-type: none"> <li>ASS1 deficiency correlated with increased tumor cell proliferation and invasion</li> </ul>	2014	Allen et al <sup>31</sup>
NCI-60 cancer cell lines	<ul style="list-style-type: none"> <li>ASS1 knockdown promotes accumulation of cytosolic aspartate for pyrimidine synthesis and increases cancer cell proliferation</li> </ul>	2015	Rabinovich et al <sup>66</sup>
HCC	<ul style="list-style-type: none"> <li>ASS1 was reduced in HCC tissues and correlated significantly to tumor stage, grade, and metastasis</li> </ul>	2017	Tao et al <sup>101</sup>
Endometrial	<ul style="list-style-type: none"> <li>ASS1 knockout downregulated DEPTOR (inhibitor of mTORC1) and therefore induced an increase in migration and invasion</li> </ul>	2017	Ohshima et al <sup>102</sup>
Colorectal	<ul style="list-style-type: none"> <li>ASS1 inhibition lowered fumarate levels (TCA cycle metabolite) leading to glycolysis rewiring</li> </ul>	2017	Bateman et al <sup>103</sup>
Glioblastoma	<ul style="list-style-type: none"> <li>ASS1 deficiency induced a rewiring of several metabolic pathways that rely on substrates generated by ASS1: <ul style="list-style-type: none"> <li>- an increase in TCA cycle metabolites</li> <li>- a decrease of intracellular levels of amino acids (alanine, glycine, threonine, serine, cysteine and tryptophan)</li> <li>- an increase of sugar metabolism (mannose, galactose or glucose)</li> </ul> </li> </ul>	2018	Mören et al <sup>104</sup>
PDAC	<ul style="list-style-type: none"> <li>A low ASS1 level is a negative prognostic biomarker for PDAC</li> </ul>	2020	Kim et al <sup>68</sup>
HCC	<ul style="list-style-type: none"> <li>Low levels of ASS1 are associated with worse overall survival</li> <li>ASS1 can act as a tumor suppressor through ER stress-induced apoptosis in mutant p53 HCC</li> </ul>	2021	Kim et al <sup>105</sup>

There was a good correlation between SCLC PDx sensitivity to pegzilarginase (4mg/kg) monotherapy and negative or low ASS1 expression by immunohistochemistry, consistent with multiple cancer cell lines studies. A clinical trial of pegzilarginase in a dose-expansion cohort of patients with SCLC has completed accrual and results are awaited (ClinicalTrials.gov Identifier: NCT03371979).

## Pegylated Arginine Deiminase

Arginine Deiminase (ADI) is an enzyme that can be found in several organisms (*Streptococcus faecalis*, *Pseudomonas putida*, *Mycoplasma hominis*, *Mycoplasma arginini*). This enzyme catalyzes the conversion of arginine into citrulline and ammonia. ADI has been formulated with polyethylene glycol (20kDa) to optimise the circulating half-life of the drug known as ADI-PEG20 (pegargininase). In 2002, Ensor et al published the first study linking ASS1 expression to the efficacy of ADI-PEG20 in melanoma and liver cancer cell lines, leading to early phase clinical trials and FDA designation of ADI-PEG20 as an orphan drug for the treatment for malignant melanoma and HCC.<sup>15,41,42</sup> ADI-PEG20 is also cytotoxic to SCLC cell lines in vitro inducing caspase-independent cell death and autophagy. SCLC xenografts were also sensitive to ADI-PEG20 (5IU) with both small and established tumors responding in an ASS1-dependent fashion on par with that seen in SCLC PDx models with pegzilarginase.<sup>21</sup>

Based on the promising preclinical data, a Phase II two-arm, non-randomised study of pegargininase enrolled 22 patients with histologically confirmed SCLC in which <5% of tumor cells expressed ASS1 by immunohistochemistry.<sup>43</sup> Cohort 1 enrolled 9 patients with “sensitive” disease who had disease control for at least 90 days after 1 previous line of

chemotherapy. Cohort 2 enrolled 13 patients with “refractory” disease who had either progressed while on chemotherapy or within 90 days of completing treatment. Both cohorts received treatment with weekly intramuscular ADI-PEG20 (320IU/m<sup>2</sup> or 36.8mg/m<sup>2</sup>) and the primary endpoint for clinical efficacy was tumor response, defined as complete response or partial response by RECIST 1.1 criteria. Although, the study was terminated early as there was poor recruitment to the sensitive disease cohort and no radiologic responses to pegargininase monotherapy, activity was identified in the refractory cohort with a patient experiencing temporary disease stabilisation and normalisation of serum LDH, a known prognostic and predictive factor in SCLC.<sup>44</sup>

## Sensitivity and Resistance to Arginine-Depleting Agents

The effects of arginine deprivation on tumor cell molecular mechanisms and the metabolome are under active investigation and vary according to cell type. Much of the mechanistic data derives from studies of ADI-PEG20 and is discussed in further detail below and in Table 2. Broadly, a number of outcomes have been reported as follows: cell cycle arrest, caspase activation, PARP cleavage, autophagy, nucleotide and polyamine metabolism rewiring, and modulation of RAS-ERK and PI3K-mTOR activity.<sup>45</sup> In SCLC, much remains to be elucidated; however, several areas are of specific interest relating to the products of arginine catabolism.

First, nitric oxide synthase (NOS) converts arginine into NO, a small reactive molecule with pleiotropic effects in host and cancer cells.<sup>46</sup> Indeed, NOS has a dominant role along with NOTCH in activating downstream soluble guanylate cyclase, a key pathway of platinum resistance in SCLC. Pharmacological depletion of NO via NOS blockade re-sensitized SCLC to chemotherapy.<sup>47</sup> NO also possesses key innate and adaptive immune signalling properties, with myeloid cell arginase – a known immunosuppressive pathway – directly competing with NOS for arginine.<sup>48,49</sup> Furthermore, inhibiting myeloid arginase and NOS has been shown to enhance tumor immunity in a variety of cancer models.<sup>50</sup> Interestingly, bronchoalveolar-derived leucocytes from patients with SCLC produce 6-fold greater levels of NO than those from patients with NSCLC.<sup>51</sup> Also, arginase-1 (ARG1) and ARG2 are expressed in SCLC in TAMs and tumor cells, respectively, with higher levels of ARG2 when compared to NSCLC, reinforcing the functional and clinical analysis of SCLC which is dependent on downstream polyamine biosynthesis.<sup>25,52–54</sup>

Apropos drug resistance, while most patients develop anti-ADI-PEG20 antibodies compared to a minority of patients developing anti-pegzilarginase antibodies, complete responses were nonetheless reported for pegargininase but none for pegzilarginase in patients with acute myeloid leukaemia.<sup>43,55,56</sup> Clearly, tumor-intrinsic rather than drug-innate mechanisms of resistance are operational with emerging rebiopsy data from several phase I/II studies providing pertinent clues. Thus, one-third of patients with the thoracic cancers mesothelioma and NSCLC progressing on pegargininase display tumoral ASS1 re-expression.<sup>30,57</sup> This is consistent with data reported using primary melanoma cell lines and also evident in patients with melanoma progressing on ADI-PEG20.<sup>58,59</sup> When ASS1 is re-expressed, cancer cells no longer depend on exogenous arginine and instead upregulate the urea cycle using citrulline generated by ADI-PEG20. Recent work by Xu et al identified contactin 1 as a mediator of rhArg-PEG (BCT-100) resistance in SCLC cell lines by promoting epithelial-mesenchymal transition linked to upregulation of AKT.<sup>60</sup>

In addition to specific alterations within tumor cells – and, notwithstanding the known limitations of obtaining rebiopsy tissue in SCLC – a critical component of resistance may be mediated by the stromal compartment. Thus, we showed ASS1-expressing macrophages highly upregulated in relapsing thoracic cancers linked to a pro-tumor chemokine network.<sup>57</sup> The role of macrophages in SCLC is being leveraged with the development of CD47 antibodies and this may provide opportunities for combination studies that are discussed further below.<sup>61</sup> Lastly, autophagy may provide a temporary and finite source of arginine for cancer cells under amino acid restriction. Both ADI-PEG20 and arginase induce autophagy in thoracic cancer cell lines, which may be modulated using antimalarial agents such as chloroquine and quinine.<sup>21,62</sup> Notably, in a recent phase I study of ADI-PEG20-based combination therapy, a patient with relapsed glioblastoma multiforme exhibited prolonged re-sensitisation to arginine deprivation following the serendipitous prescription of quinine for leg cramps.<sup>63,64</sup> The clinical implications of autophagy modulators in the context of arginine deprivation for cancer therapy will require further study.

**Table 2** Functional and Metabolic Effects of ADI-PEG20

Cancer Type	Functional/Metabolic Effect	Year	Study
Osteosarcoma Neuroblastoma	<ul style="list-style-type: none"> <li>• ADI inhibits the proliferation of vascular endothelial cells</li> <li>• ADI inhibits proliferation by cell cycle arrest in G<sub>1</sub> and/or S phase leading to apoptosis</li> </ul>	1999	Gong et al <sup>106</sup>
Retinoblastoma Sarcoma	<ul style="list-style-type: none"> <li>• ADI-PEG20 depletes plasma arginine (from 155µm to 2 µm)</li> <li>• ADI-PEG20 inhibits the production of NO</li> </ul>	2002	Thomas et al <sup>107</sup>
Melanoma HCC	<ul style="list-style-type: none"> <li>• ADI specifically depletes arginine</li> <li>• ADI-PEG20 induces significant inhibition of tumor growth in vivo and increases survival of mice</li> </ul>	2002	Ensor et al <sup>15</sup>
Mesothelioma	<ul style="list-style-type: none"> <li>• ADI-PEG20 induces BAX conformational change alongside mitochondrial inner membrane depolarization in ASS1 deficient mesothelioma cells</li> </ul>	2006	Szlosarek et al <sup>16</sup>
Melanoma	<ul style="list-style-type: none"> <li>• ADI-PEG20 treatment induces apoptosis by PARP cleavage</li> </ul>	2007	Savaraj et al <sup>108</sup>
PDAC	<ul style="list-style-type: none"> <li>• ADI-PEG20 inhibited the growth of ASS1 deficient PDAC cell lines</li> <li>• ADI-PEG20 induced caspase activation and apoptosis</li> </ul>	2008	Bowles et al <sup>109</sup>
PDAC	<ul style="list-style-type: none"> <li>• ADI-PEG20 triggers autophagy as a protective response mechanism</li> <li>• ADI-PEG20 inhibits tumor growth in vivo</li> </ul>	2009	Kim et al <sup>110</sup>
Melanoma	<ul style="list-style-type: none"> <li>• ADI-PEG20 treatment upregulates death receptor expression</li> </ul>	2010	You et al <sup>111</sup>
SCLC	<ul style="list-style-type: none"> <li>• ADI-PEG20 treatment induced autophagy and caspase-independent cell death</li> <li>• ADI-PEG20 treatment induced significant inhibition of tumor growth in vivo</li> </ul>	2012	Kelly et al <sup>21</sup>
Head and Neck	<ul style="list-style-type: none"> <li>• ADI-PEG20 inhibits the proliferation of head and neck cancer cells</li> </ul>	2012	Huang et al <sup>112</sup>
Melanoma Breast cancer Lymphoma	<ul style="list-style-type: none"> <li>• Upon long term of ADI-PEG20 treatment, resistance appears due to ASS1 upregulation through c-MYC</li> <li>• ADI-PEG20 activates RAS signalling, ERK and the PI3K/AKT/GSK-3β kinase cascade that suppress c-MYC expression overtime</li> </ul>	2012	Tsai et al <sup>113</sup>
Melanoma	<ul style="list-style-type: none"> <li>• ADI-PEG20 treatment of melanoma cells induces a decrease in ATP levels and inactivates the mTOR pathway triggering autophagy. Arginine is a key regulator of mTOR (with leucine) and therefore depleting arginine modulates mTOR levels</li> </ul>	2013	Long et al <sup>22</sup>
Mesothelioma	<ul style="list-style-type: none"> <li>• ADI-PEG20 resistance is mediated via the demethylation of ASS1 promoter and not via c-MYC</li> <li>• ADI-PEG20 resistance is linked to the rewiring of polyamine metabolism</li> <li>• Polyamine levels are depleted upon ADI-PEG20 treatment in ASS1 deficient mesothelioma cell lines whereas it remained unchanged in ADI-PEG20 resistant cells</li> </ul>	2016	Locke et al <sup>24</sup>
Glioblastoma	<ul style="list-style-type: none"> <li>• ADI-PEG20 treatment triggers cellular senescence with cells undergoing morphological changes such as vacuolisation, flattening, accumulation of stress granules and β-galactosidase expression</li> </ul>	2017	Maletzki et al <sup>114</sup>
PDAC	<ul style="list-style-type: none"> <li>• ASS1-deficient cells upregulated asparagine synthetase and rewiring of aspartate use inducing nucleotide insufficiency and thus impairing the cell cycle upon ADI-PEG20 treatment</li> </ul>	2020	Kim et al <sup>68</sup>
Melanoma Leiomyosarcoma	<ul style="list-style-type: none"> <li>• ADI-PEG20 treatment induced glutamine anaplerosis and upregulation of oxidative phosphorylation</li> </ul>	2017	Kremer et al <sup>27</sup>

## Strategic Combinations Incorporating Arginine Deprivation for SCLC Therapy

The disconnect between the marked preclinical efficacy of arginine deprivation in SCLC and an apparent lack of response of ADI-PEG20 monotherapy in patients may be explained by several factors, including tumor adaptation by ASS1 re-expression and autophagy, stromal refuelling and tumor heterogeneity. To overcome these hurdles, combination

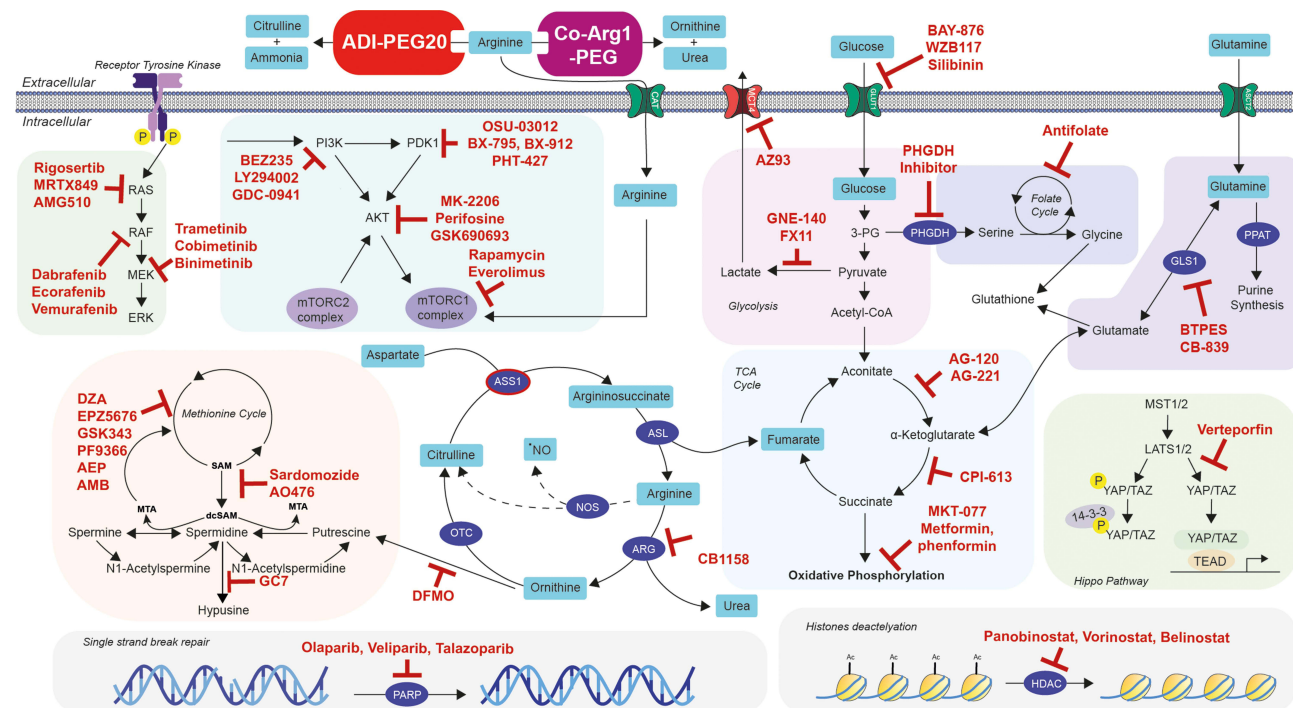
treatments of ADI-PEG20 have now progressed into phase II and III studies in multiple cancers, and here we explore how these might be configured for SCLC as summarised in Figure 1.

### Targeting DNA Repair

PARP are enzymes that play an important role in DNA repair by transferring ADP-ribose residues to targeted proteins. Some tumors depend highly on PARP for survival and therefore PARP inhibition is an interesting therapeutic strategy for these cancers.<sup>55</sup> PARP inhibitors (olaparib, veliparib, talazoparib) have been investigated for SCLC treatment. In 2019, Van Den Borg et al showed promising preliminary evidence of the preclinical and clinical activity of these molecules for SCLC treatment especially in sensitising cells to chemotherapy.<sup>65</sup> Moreover, in lymphoma, ADI-PEG20 treatment induced the activation of PARP cleavage, a marker of apoptosis, suggesting that arginine deprivation can have an impact on PARP derived pathways.<sup>19</sup> This highlights the potential for enhanced cytotoxicity by targeting the DNA damage/repair pathway with arginine deprivation in SCLC.

### Targeting DNA Synthesis and Replication

The high proliferative capacity of SCLC and the consequent demand for increased nucleotide precursors represents an area of potential combination studies with arginine deprivation. We showed that ADI-PEG20 downregulated thymidine salvage and de novo synthesis as a mechanism underlying potentiation with the anti-folate pemetrexed in ASS1-deficient mesothelioma and bladder cell lines and consistent with the known diversion of aspartate for enhanced nucleotide synthesis in arginine auxotrophs.<sup>31,66</sup> Previous work in HCC cell lines revealed potentiation by arginase with the thymidylate synthase inhibitor 5-fluorouracil.<sup>67</sup> In addition to pemetrexed, ADI-PEG20 also synergised with the histone deacetylase inhibitor panobinostat in ASS1 deficient pancreatic ductal adenocarcinoma (PDAC).<sup>68</sup> The synthetic lethality was explained by the combination of accumulating DNA damage from panobinostat and cell cycle S-phase arrest upon ADI-PEG20 treatment in ASS1 deficient PDAC. In ASS1-ve cancer models, Prudner et al



**Figure 1** Future strategic combinations of arginine deprivation with personalised targeted therapeutics for SCLC management. **Notes:** Schematic showing the main metabolic and signalling pathways linked to arginine biosynthesis and metabolism (glycolysis, TCA cycle, serine metabolism, glutaminolysis, polyamine metabolism, hippo pathway, PAM pathway, RAS/RAF/MEK/ERK pathway, single strand break repair, and histones deacetylation pathway). ADI-PEG20 and Co-Arg1-PEG are two molecules able to convert external arginine into citrulline and ammonia or ornithine and urea, respectively. Molecules denoted in red indicate potential drug candidates in combination with arginine deprivation for the management of SCLC.

highlighted the efficacy of the combination of ADI-PEG20 with docetaxel and gemcitabine both in vitro and in vivo. The study suggests that ADI-PEG20 with docetaxel translocate c-MYC to the nucleus thereby promoting hENT1 expression which induces gemcitabine uptake and increased cell death.<sup>69</sup> An ongoing phase II clinical trial is investigating the potential of this therapeutic combination for the treatment of sarcoma and SCLC (NCT03449901). Moreover, pemetrexed, gemcitabine and panobinostat have been studied in preclinical and clinical settings in SCLC raising the possibility of further combinations with arginine deprivation.<sup>70–73</sup> Similarly, arginine deprivation also modulates platinum and temozolomide cytotoxicity with evidence for additive and synergistic effects in arginine-auxotrophic cell lines.<sup>17,74</sup> While platinum forms the backbone of SCLC chemotherapy, temozolomide has documented activity in relapsed disease and further studies are warranted in combination with ADI-PEG20 and rhArgPEG.<sup>75</sup>

## Polyamines

Polyamines are highly regulated positively charged organic molecules that are found to be dysregulated in various cancers. Polyamines are essential for several cellular functions such as cell proliferation, apoptosis regulation, gene expression and protein translation. However, the mechanisms underlying these effects are yet to be fully described and understood. Locke et al showed the rewiring of the polyamine metabolism pathway in mesothelioma upon the appearance of ADI-PEG20 resistance.<sup>24</sup> Furthermore, combination treatment with a polyamine inhibitor reversed resistance to ADI-PEG20. Chalishazar et al highlighted the importance of the polyamine pathway in MYC-driven SCLC which was highly dependent on arginine regulation and sensitive to the polyamine biosynthesis inhibitor DFMO.<sup>25</sup> Efforts are ongoing to understand the role of the polyamine pathway in arginine auxotrophic cancers with the aim of developing novel combinations for testing in the clinic, including for patients with SCLC.

## Glutamine Metabolism

Phosphoribosyl pyrophosphate amidotransferase (PPAT) transfers the gamma-nitrogen atom of glutamine to 5-phosphoribosyl pyrophosphate as the rate-limiting step of purine synthesis. RNA-sequencing of patients with SCLC revealed that PPAT expression was upregulated in cancerous cells compared to normal tissue whereas glutaminase was downregulated (GLS1).<sup>76</sup> These results indicate a rewiring of glutamine metabolism in SCLC and highlight the importance of this pathway. In melanoma and sarcoma, arginine deprivation induced by ADI-PEG20 caused a compensatory upregulation of the anaplerotic glutamine-glutamate axis refuelling the TCA cycle. This dependence was confirmed by the combination of ADI-PEG20 with GSL1 inhibitor BTPES that showed a synthetic lethal effect in these cell models.<sup>22,27</sup> Thus, the potency of a combination of ADI-PEG20 with a glutamine metabolism inhibitor is of interest in ASS1 deficient SCLC, thereby assessing the extent of glutamine metabolism rewiring upon arginine deprivation.

## Hippo Pathway

Recent studies suggest four different molecular subtypes of SCLC depending on their neuroendocrine differentiation and gene expression profile.<sup>5</sup> SCLC-Y is driven by the expression of YAP1, a key regulator of the Hippo pathway. TEAD transcription factors mediate YAP1 activity to promote cell proliferation and growth. In SCLC, the YAP1 subtype is associated with a better prognosis compared to the other subtypes.<sup>77</sup> Several molecules, including verteporfin, decursin, amlexanox, and latrunculin, are known to inhibit YAP1-mediated transcription. Recent work in prostate cancer cells indicated that blocking TEAD activity potentiated the effect of arginine deprivation thus expanding these studies to SCLC are warranted.<sup>78</sup>

## PI3K/AKT/mTOR (PAM) Pathway

The PAM pathway is frequently dysregulated and active in SCLC in line with several other cancers. This pathway promotes cell growth and proliferation (via mTORC1) and cell cycle progression (via CDK4) while inhibiting apoptosis (via caspase-3). Various strategies are under development to target the recurrent alterations of this pathway

in cancer, including inhibitors of the PAM pathway in combination with arginine deprivation, with clear applicability to SCLC.<sup>79</sup>

## Glycolysis and Warburg Effect

In SCLC, MYC-driven cancers display upregulated expression of glycolysis genes, correlating with a robust Warburg effect whereby the MYC-driven cells are less reliant on oxidative metabolism compared to cells with low MYC expression.<sup>80</sup> The use of glycolysis inhibitors such as PFK158 showed a significant decrease in glucose uptake, ATP production, and lactate for the MYC-driven cells, and controlled tumor growth *in vivo*.<sup>80</sup> ADI-PEG20 inhibited oxidative phosphorylation and aerobic glycolysis, therefore effectively inhibiting the Warburg effect in melanoma and sarcoma cell lines. Hence, further testing of ADI-PEG20 with inhibitors of glycolysis may provide a novel metabolic combination strategy for MYC-driven SCLC.

## Immunotherapy Combinations

SCLC is a highly mutated cancer with a history of heavy tobacco use in 98% of patients, the highest for any malignancy. Consequently, SCLC is immunologically tractable with recent studies defining a role for programmed death-ligand 1 (PD-L1) antagonists in combination with chemotherapy.<sup>1</sup> However, despite the high tumor mutation burden, the benefits of immune checkpoint therapy have been modest, implying a role for extensive tumor-induced immunosuppression. Recent single cell transcriptome data have revealed marked T cell dysfunction – a low ratio of CD8<sup>+</sup> effector to Treg cells – and enrichment of profibrotic macrophages linked to exhausted CD8<sup>+</sup> T cells as major drivers of the immune cold TME.<sup>81</sup>

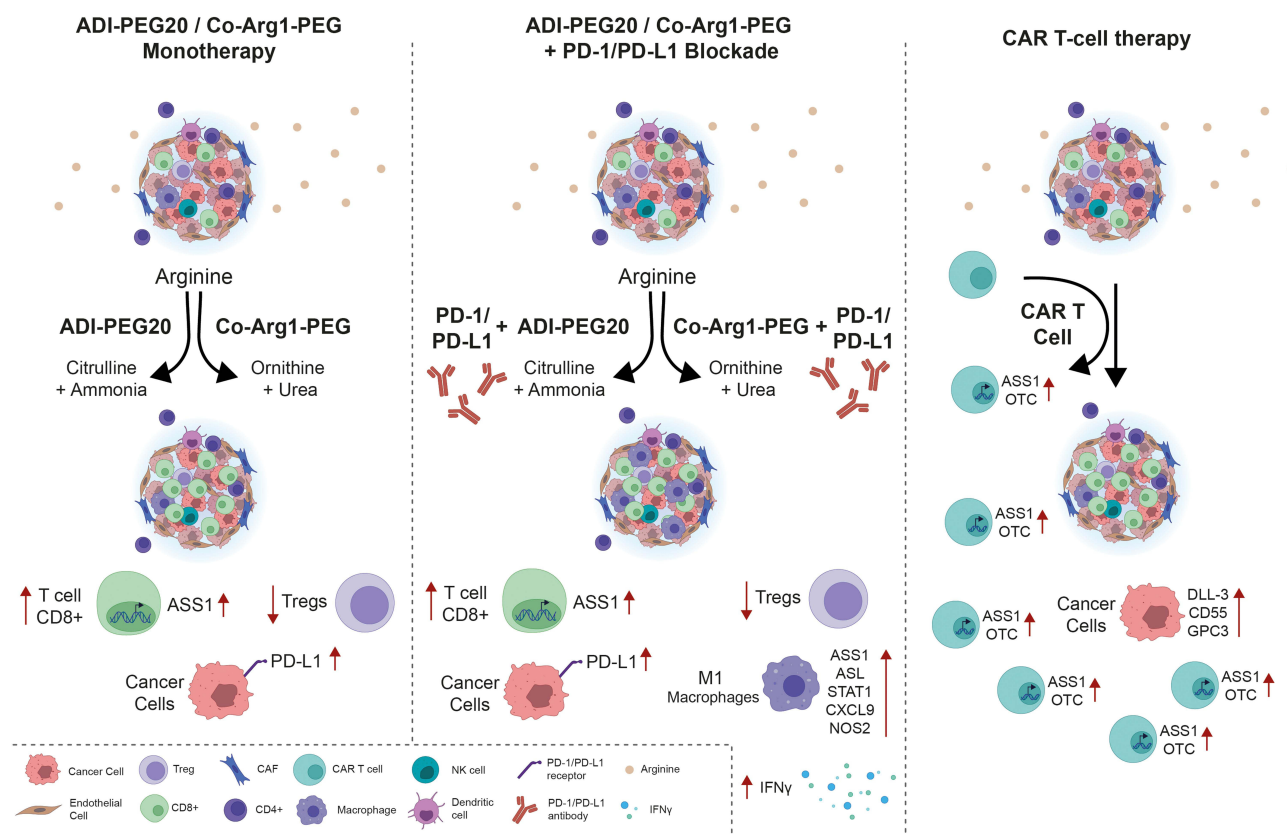
An anticancer immune response is dependent on multiple factors, with arginine playing pivotal roles especially in maintaining T cell receptor function, proliferation and memory.<sup>82</sup> Critically, nitric oxide synthase (NOS) via the generation of NO, and arginase (1 and 2) via the production of ornithine, are major arginine catabolizing enzymes involved in orchestrating cellular crosstalk and thereby immune and inflammatory responses.<sup>83,84</sup> Recently, low plasma levels of arginine have been linked to poor outcomes in patients receiving immune checkpoint inhibitors, whereas high circulating arginine was linked to improved disease control, further emphasizing the potential of arginase inhibitors as cancer therapeutics.<sup>85</sup> Nonetheless, preclinically, there is also a good rationale for combining immune checkpoint immunotherapy with arginine deprivation as shown in [Figure 2](#). ADI-PEG20 increases tumoral PD-L1 expression and T cell infiltration and reduces Treg cell accumulation with potentiation of PD-1/PD-L1 inhibitors in arginine auxotrophic syngeneic tumor murine models.<sup>86</sup> Similarly, Co-Arg1-PEG has shown enhanced tumor control *in vivo* in combination with PD-1/PD-L1 inhibitors and anti-OX40 antibodies.<sup>87</sup> Moreover, analysis of urea cycle dysfunctional cancer predicts for gene signatures linked to immune responsiveness.<sup>88</sup> Additionally, targeted antigens for chimeric antigen receptor (CAR) T-cell-based therapy in preclinical and clinical studies of SCLC, namely DLL-3, CD55 and GPC3, may be enhanced through metabolic engineering of urea cycle enzymes, ASS1 and OTC, thereby maintaining T cell immunocompetency.<sup>89</sup> Additionally, citrulline the byproduct of ADI-PEG20, may be recycled by T cells to regenerate arginine thereby maintaining immune cell function, a critical consideration with immune checkpoint inhibitors.<sup>90</sup>

Clinically, ADI-PEG20 was tested with pembrolizumab in a small phase 1b study of patients with advanced treatment-refractory cancers, mostly aerodigestive in origin, and none with lung cancer.<sup>91</sup> The combination was safe and active, documenting a partial response rate of 24% and a grade 3–4 neutropenia rate of 40%, which is notable in that anti-PD-1 inhibitors rarely cause neutropenia and this occurs infrequently with ADI-PEG20 monotherapy.<sup>29,41,42,59</sup> Repeat biopsies on ADI-PEG20 – and prior to commencement of pembrolizumab – revealed infiltration by CD3<sup>+</sup> T cells consistent with the pre-clinical data. Results are also expected from a phase I/II study of pegzilarginase combined with pembrolizumab which included a SCLC dose-expansion cohort (ClinicalTrials.gov Identifier: NCT03371979).

## Current Therapy for Small Cell Lung Cancer and Outcomes

Whilst in general SCLC responds robustly to initial platinum-based chemotherapy, the majority of patients subsequently relapse.<sup>92,93</sup> The chance of responding to second-line treatment is approximately 10% and resistance emerges rapidly in





**Figure 2** Potential immunometabolic combinations incorporating arginine deprivation for the management of SCLC.

**Notes:** Schematic illustrating how arginine deprivation may potentiate existing immune checkpoint inhibitors and cellular (CAR-T cell) immunotherapies with applicability to SCLC.

nearly all patients.<sup>94</sup> Clinically, the terms “limited disease”, and “extensive disease” are used to define the extent of SCLC burden. Limited disease is defined as the tumor being confined to one hemithorax and regional lymph nodes and treatment options include surgical resection in a minority aiming for R0 resection, concurrent chemotherapy (with platinum-based chemotherapy in combination with the topoisomerase II inhibitor, etoposide) and thoracic radiotherapy. Prophylactic cranial irradiation (PCI) serves to decrease the risk of symptomatic brain metastases and increases overall survival in patients who are Performance status 0–1 and experience a complete remission.<sup>95</sup> Despite this, the median OS of limited-stage SCLC is approximately 17 months.

Patients with “extensive disease” are treated with platinum-based chemotherapy usually combined with etoposide with a resultant median overall survival (OS) of 9–10 months, progression free survival (PFS) of 5–6 months and 1 year OS of ~35%.<sup>96</sup> A modest advance in the last 40 years in the first-line treatment setting of SCLC has been the advent of chemoimmunotherapy with the addition of an anti-programmed death-ligand 1 (PD-L1) antibody based on the IMpower133 and CASPIAN studies.<sup>97–99</sup> The IMpower 133 trial tested the addition of the anti-PD-L1 antibody, atezolizumab to carboplatin and etoposide for 4 cycles followed by atezolizumab maintenance versus placebo and carboplatin and etoposide. Patients in the atezolizumab arm achieved a median OS of 12.3 months compared to 10.3 months in the placebo arm (HR = 0.70; 95% CI 0.54 to 0.91 p = 0.007) and with almost 2 years of median follow-up, patients in the atezolizumab arm continued to display benefit with ~22% of patients alive at 24 months in the atezolizumab arm, compared with ~14% in the placebo arm.<sup>97</sup>

The CASPIAN trial enrolled 805 patients to durvalumab with or without the anti-cytotoxic T-cell lymphocyte-4 blocking antibody, tremelimumab, plus platinum-etoposide followed by durvalumab maintenance or platinum-etoposide only. The combination of platinum-etoposide plus durvalumab significantly extended OS, compared with platinum-etoposide alone (HR = 0.73) 95% CI, CI 0.59 to 0.91; p = 0.0047. Furthermore, patients in the durvalumab/platinum/

etoposide arm had a sustained OS benefit; an additional 8% of patients were alive at 24 months in the experimental arm (22.2%) compared with the platinum/etoposide arm (14.4%). The addition of tremelimumab provided no additional survival benefit.<sup>98</sup> While KEYNOTE-604, which tested the role of pembrolizumab with platinum-etoposide, significantly improved PFS, the pre-specified endpoint for OS was not met.<sup>100</sup> Despite these recent therapeutic developments with the addition of anti-PD-L1-based immunotherapy to standard chemotherapy for SCLC, the prognosis remains poor and new strategies are clearly required to go beyond the current therapeutic plateau.

Thus, in view of the modest gains obtained by incorporating anti-PD-L1 blockade to the carboplatin-etoposide chemotherapy backbone, there is clear scope for further improving survival outcomes for patients with SCLC. Arginine deprivation is a particularly attractive anti-metabolite option that on current evidence is safe in triplet chemotherapy combinations and as a doublet with immune checkpoint blockade. Indeed, in view of the preclinical data described above, there are potential synergies to explore for both the chemotherapy and immunotherapy components as a quadruplet chemo-immunometabolic therapy with arginine depletion.

## Perspectives for Arginine Deprivation in SCLC

ATOMIC-meso is the first front-line triplet chemotherapy phase III study of arginine deprivation using ADI-PEG20 combined with platinum and pemetrexed in cancer, specifically non-epithelioid mesothelioma, and is expected to report by the summer of 2022. Based on the premise that ADI-PEG20 sensitises ASS1-deficient cancers to antifolates by exploiting a critical dependency on nucleotide synthesis, ATOMIC-meso, if positive, will herald the advent of arginine deprivation as a novel antimetabolite therapy for cancer.

As alluded to earlier, there is significant potential to leverage arginine auxotrophic SCLC with arginine deprivation in combination with chemoimmunotherapy, namely platinum plus etoposide and an anti-PD-L1 agent which would be a rational pathway to development. Other combinations may have merit particularly replacing etoposide for pemetrexed in patients with reduced performance status. Efforts are also underway with a phase II trial investigating ADI-PEG20 in combination with gemcitabine and docetaxel, that includes a small cohort of patients with SCLC. (NCT03449901). Additional agents that warrant investigation in relapsed SCLC based on a study in progress in glioblastoma multiforme, include ADI-PEG20 with temozolomide with or without radiation (NCT04587830).

## Conclusions

There are significant opportunities ahead to develop our understanding of the role of arginine deprivation as part of combined multimodality care for patients with SCLC. More broadly, arginine depletion as an antimetabolite strategy should be reinterpreted both in cancer treatment and in reshaping the tumor microenvironment, with a specific focus on tumor-type. Ultimately, a strong translational base will be needed to decipher which SCLC subgroups benefit the most from incorporating arginine depletion to the standard of care.

## Disclosure

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