

Methods of Treating Cancer and Combinations Thereof

Field of Invention

The present invention relates generally to the field of oncology. In particular, the specification teaches a method of treating cancer or reducing the risk of recurrence of cancer in a subject.

Background

Metabolic alteration is a cancer hallmark that provides for crucial functional adaptations along the course of disease progression. The ability of tumor cells to rewire cellular metabolism to meet metabolic demands such as increased dependency on glucose and glycolytic flux, upregulation of glutaminolytic reactions and nucleotide synthesis, and utilization of lactate as alternate carbon source for proliferation is seen in most, if not all, human tumors. These alterations are intricately linked to sustaining tumor proliferation and invasive capacity of tumor cells, as well as maintaining stemness in certain tumor types. Otto Warburg first discovered that tumor cells exhibited high rates of aerobic glycolysis and proposed impaired oxidative phosphorylation (OXPHOS) as the underlying cause for the phenomenon. However, mitochondrial OXPHOS defects are rare in most cancer type; isotope-tracing experiments performed in several cancers demonstrated unequivocally that glucose was oxidized to carbon dioxide in the tumor cells. Quite strikingly, tumor cells display metabolic plasticity in maintaining both glycolysis and OXPHOS for adapting to fluctuating tumor microenvironmental conditions. Under normoxia, high rates of aerobic glycolysis and OXPHOS provide biosynthetic precursors and ATP to fuel anabolic processes essential for rapid cell growth and proliferation. On the other hand, hypoxia-inducible factor 1 (HIF-1) may drive upregulation of glucose transporters and various glycolytic enzymes to enhance glycolytic rate at the cost of suppressed OXPHOS during hypoxia.

Other than rewiring glucose metabolism, non-small cell lung cancer tumors have been shown to harness lactate as a carbon source to drive tricarboxylic acid (TCA) cycle *in*

vivo. Lactate, a byproduct of glycolysis, is secreted by cancer cells via monocarboxylate transporters (e.g. MCT1 and MCT4), when pyruvate is reduced via lactate dehydrogenase (LDH). This recycles NAD⁺ required for further rounds of glycolysis and maintains intracellular pH of cells. However, lactate can also function as inter-organ carbon shuttle at the organism level, and as an oncometabolite provided by stromal cells to fuel TCA cycle and lipogenesis in tumor cells. Such nutrient exchanges between cell types with different metabolic signatures, particularly in tumors which display intra-tumoral heterogeneity, could help them overcome nutrient limitations in tumor microenvironment. Through autophagic response in stroma-associated pancreatic stellate cells, pancreatic ductal adenocarcinoma (PDAC) could acquire sufficient alanine to fuel TCA cycle and lipid biosynthesis in glucose-limiting and serum-derived nutrient-limiting tumor microenvironment. The ability to alter nutrient requirement and scavenge the microenvironment for alternate carbon sources is, therefore, an important metabolic adaptation of malignant cells.

Tumor-initiating cells (TICs), referred as cancer stem cells in certain context, represent a subpopulation of neoplastic cells that drives tumor-initiation and contribute towards intratumoral heterogeneity, thereby resulting in drug resistance and cancer relapse. TICs have been found to be metabolically distinct from bulk tumor cells, in part, through elevated dependency on exogenous methionine. They also appeared to be less glycolytic compared to their differentiated counterparts and have lower steady state amounts of glycolytic substrates. Given that TICs tend to reside in more hypoxic or poorly vascularized regions of tumors, this raises questions on the metabolic plasticity that might be adopted to overcome microenvironmental stress. For instance, TICs can activate HIF1 α and drive PKM2 expression for robust glycolysis to overcome low oxygen tension. Hypoxic cancer cells can also have diminished mitochondrial acetyl-CoA production from glucose and instead rely on exogenous glutamine and acetate for acetyl-CoA production to drive lipid biosynthesis. However, the manner by which they adapt to periods of low nutrient availability, especially during glucose-limiting conditions, has remained unclarified.

Accordingly, it is generally desirable to overcome or ameliorate one or more of the above-mentioned difficulties.

Summary

Disclosed herein is a method of treating cancer or reducing the risk of recurrence of cancer in a subject, the method comprises contacting a CD147 overexpressing cell with an inhibitor of Monocarboxylate Transporter 1 (MCT1) to treat the cancer in the subject.

The CD147 overexpressing cell may be a tumor initiating cell.

Disclosed herein is a method of treating cancer or reducing the risk of recurrence of cancer in a subject, wherein the method comprises inhibiting a CD147 overexpressing cell with an inhibitor of MCT1 to treat the cancer or reduce the risk of recurrence of cancer in the subject.

Disclosed herein is a method of treating cancer or reducing the risk of recurrence of cancer in a subject, wherein the method comprises administering to the subject an inhibitor of MCT1 to treat the cancer or reduce the risk of recurrence of cancer in the subject, wherein the cancer is associated with overexpression of CD147 in one or more tumor initiating cells.

Disclosed herein is an inhibitor of MCT1 for use in treating cancer or reducing the risk of recurrence of cancer in the subject, wherein the cancer is associated with overexpression of CD147 in one or more tumor initiating cells.

Disclosed herein is the use of an inhibitor of MCT1 in the manufacture of a medicament for treating cancer or reducing the risk of recurrence of cancer in the subject, wherein the cancer is associated with overexpression of CD147 in one or more tumor initiating cells.

Disclosed herein is a method of detecting a cancer that is likely to be susceptible to treatment with an inhibitor of MCT1, the method comprising detecting increased level of expression of CD147 in a sample obtained from the subject, wherein an increased level of expression of CD147 as compared to a reference identifies the subject as one who is suitable for treatment with an inhibitor of ketone metabolism.

Disclosed herein is a method of treating cancer or reduce the risk of recurrence in a subject, the method comprising: a) detecting increased level of expression of CD147 in a sample obtained from the subject, wherein an increased level of expression of CD147 as compared to a reference identifies the subject as one who is suitable for treatment with an inhibitor of MCT1, FASN or CD147, and b) administering an inhibitor of MCT1 to the subject so as to treat the cancer or reduce the risk of recurrence of cancer in the subject.

Disclosed herein is a method of treating cancer or reduce the risk of recurrence in a subject, the method comprising: a) selecting a subject having increased level of expression of CD147 in a sample obtained from the subject, wherein an increased level of expression of CD147 as compared to a reference identifies the subject as one who is suitable for treatment with an inhibitor of MCT1, FASN or CD147, and b) administering an inhibitor of MCT1 to the subject so as to treat the cancer or reduce the risk of recurrence of cancer in the subject.

Disclosed herein is a method of stratifying a subject into one who is likely to be suffering from an aggressive or a non-aggressive cancer, the method comprising analyzing the level of CD147 expression in a sample that is obtained from the subject, wherein i) an increased level of expression of CD147 as compared to a reference identifies the subject as one who is likely to be suffering from an aggressive cancer and ii) a lack of increase of expression of CD147 identifies the subject as one who is likely to be suffering from a non-aggressive cancer.

Disclosed herein is a method of predicting a likelihood of a subject who is suffering from an aggressive cancer, wherein the method comprises detecting increased level of expression of CD147 in a sample obtained from the subject, wherein an increased level of expression of CD147 in the sample as compared to a reference indicates that the subject is likely to be suffering from an aggressive cancer.

Brief Description of Drawings

Embodiments of the present invention are hereafter described, by way of non-limiting example only, with reference to the accompanying drawings in which:

Figure 1. Adaptations of lung TICs and in-vivo effects of acetoacetate supplementation under glucose-limiting conditions. **A**, Quantification of basal and maximal (Left) ECAR and (Right) OCR in lung TICs and Adh cells. ECAR and OCR levels were normalized to cell count or DAPI intensity (n = 3 per group for left, n = 9 per group for right). **B**, Proliferation assay of lung TICs and Adh cells in 5.5 or 25mM glucose. **C**, Proliferation assay of lung TICs in 0, 1.4, 5.5 or 25mM glucose. Readings were determined by normalizing luminescence reading to day 0. **D**, (Left) Heatmap shows top 100 differentially expressed genes (FDR < 0.05) in lung TICs cultured under ultra-low glucose condition (ULG; 1.4mM) or high glucose condition (HG; 25mM). (Right) Zoomed in heatmap shows metabolic genes of interest grouped according to known metabolic pathways. **E**, (Left) Schematic illustrates stepwise conversion of β -hydroxybutyrate to acetyl-CoA. Enzymes catalyzing each step are in red. (Right) BDH1, OXCT1 and ACAT2 transcript levels of lung TICs quantified by qPCR. Transcript levels in ULG condition are normalized to lung TICs cultured in HG condition. **F**, Proliferation assay of lung TICs under ULG or ULG with acetoacetate conditions. Readings were determined by normalizing luminescence reading to day 0 (n = 10 per group for **B**, **C** and **F**). **G**, Growth of lung TICs xenografts derived from viable cells harvested on Day 4 (from **F**) subcutaneously injected into flanks of NSG mice (n = 3 mice). **H**, Growth of lung TICs and Adh cells xenografts subcutaneously injected into flanks of NSG mice fed with standard or ketogenic diet (n = 4 mice for standard diet and n = 8 mice for ketogenic diet). **I**, Growth of repopulated lung TICs xenografts derived from viable cells of TICs tumor excised from flanks of NSG mice fed with standard diet or ketogenic diet (n = 4 mice) (from **H**). Statistical significance in **A-C**, **E-I** are determined by unpaired two tailed t-test. (* p < 0.05, ** p < 0.01, *** p < 0.001, p < 0.0001, n.s = not statistically significant).

Figure 2. LC-MS reveals ketone body supplementation increases levels of ketolytic and TCA cycle intermediates, and fatty acids. **A**, Heatmap depicts top 20 up and downregulated metabolites (FDR < 0.05) from global LC-MS profiling in lung TICs cultured under ULG or ULG with acetoacetate conditions. Metabolites were grouped

according to known metabolic pathways. **B**, Intracellular metabolite levels in lung TICs cultured under ULG with acetoacetate condition were quantitated by LC-MS and normalized to lung TICs cultured under ULG condition (n = 6). **C**, Schematic illustrates metabolic pathway for conversion of labelled β -hydroxybutyrate to labelled citrate which fuels *de novo* lipogenesis. Enzymes catalyzing each step are in red. **D**, Intracellular ^{13}C -labelled β -hydroxybutyrate or **E**, ^{13}C -labelled citrate levels at 30 min and 24 hours in lung TICs cultured under ULG condition supplemented with ^{13}C -labelled β -hydroxybutyrate were quantitated by LC-MS and normalized to lung TICs cultured under ULG condition at 0 min (n = 9 for **D** and **E**). **F**, Intratumoral metabolites (non-fatty acids) and **G**, fatty acid levels in lung TICs xenografts derived from NSG mice fed with standard or ketogenic diet. Metabolite levels were quantitated by LC-MS and normalized to readings from standard diet (n = 6 for **F** and **G**). Statistical significance in **B**, **D-G** are determined by unpaired two tailed t-test. (* p < 0.05, **, p < 0.01, *** p < 0.001, n.s = not statistically significant).

Figure 3. MCT1 inhibition using clinically advanced inhibitors can target lung TICs by inhibiting ketone transport. **A**, (Left) Schematic illustrates uptake and metabolism of ketone bodies in cells. Enzymes or transporters catalyzing each step are in red. (Right) Western blot shows staining of enzymes and transporters involved in ketone body metabolism illustrated in (Left) from the whole cell lysates of lung TICs and Adh cells. Beta-actin serves as loading control. **B**, Transcript levels of lipid metabolism and related genes from lung TICs quantified by qPCR. Transcript levels of lung TICs are normalized to transcript levels of Adh cells (n = 3). **C**, Dose-response inhibition curves depict the effect of cerulenin on lung TICs cultured in varying conditions. **D**, Proliferation assay of lung TICs cultured in ULG condition with acetoacetate supplemented and treated with vehicle or 3 μM cerulenin (n = 9). **E**, Growth of lung TICs xenografts subcutaneously injected into flanks of NSG mice fed with standard (n = 4) or **F**, ketogenic diet (n $\geq$ 4 mice). Mice were subjected to oral gavage with either corn oil or cerulenin at 30mg/kg per 3-day intervals when tumor size $\geq$ 5mm at Day 14 (n = 6 for corn oil and n = 4 for cerulenin). **G**, Western blot shows staining of proteins involved in ketogenesis from whole cell lysates of co-cultured lung TICs and Adh cells cultured under HG or ULG conditions. Beta-actin serves as loading control. **H**, Schematic illustrates metabolic pathway for conversion of beta-hydroxybutyrate to

acetyl-CoA. Enzymes catalysing ketolysis are in green, while enzymes catalysing ketogenesis are in red. **I**, mRNA levels of genes involved in ketolysis (in green) and ketogenesis (in red) from Adh cells cultured under ULG condition are quantified by qPCR and normalized to transcript levels of Adh cells cultured under HG condition. **J**, Dose-response inhibition curves depict the effect of AZD3965 MCT1 inhibitor on lung TICs cultured in varying conditions. **K**, Proliferation assay of lung TICs cultured in ULG condition with acetoacetate supplemented and treated with vehicle or 3 μ M AZD3965 (n = 9). **L**, Growth of lung TICs xenografts subcutaneously injected into flanks of NSG mice fed with standard (Std) or ketogenic (Keto) diet (n = 6 mice). Mice were subjected to oral gavage with either corn oil (Veh) or AZD3965 (AZD) at 50mg/kg per 2-day intervals when tumor size $\geq$ 5mm on Day 14. **M**, Representative confocal images of BODIPY⁺ staining in cryosections of tumor sections derived from lung TICs xenografts excised from NSG mice fed with standard (Std) or ketogenic (Keto) diet and treated with corn oil (Veh) or AZD3965 (AZD). **N**, Quantified numbers of lipid droplets in **M** were determined by ImageJ (n = 3). Relative cell viability of all dose-response inhibitor curves in **C** and **J** were normalized to their respective DMSO control. Data was fitted to four-parameter dose(log) inhibitor analysis and IC₅₀ was determined. Both proliferation assay readings in **D** and **K** were determined by normalizing luminescence reading to day 0. Statistical significance in **B**, **D-F**, **K-L**, **N** are determined by unpaired two tailed t-test. (* p < 0.05, ** p < 0.01, *** p < 0.001, p < 0.0001, n.s = not statistically significant).

Figure 4. MCT1 inhibition using clinically advanced inhibitors can target lung TICs by inhibiting ketone transport. **A**, Western blot shows staining of CD147 and MCT1 from the whole cell lysates of CD147 or MCT1 knockout lung TICs. Beta-actin serves as loading control (n = 3). **B**, Transcript levels of MCT1 in control or CD147 KO lung TICs quantified by qPCR. Transcript levels are normalized to transcript levels of control KO lung TICs. **C**, Multiplex immunofluorescence staining of CD147, MCT1 and DAPI on control, CD147 KO and MCT1 KO lung TICs; Scale bar represents 100 μ M. **D**, Western blot shows staining of MCT1 from the whole cell lysates of CD147 KO lung TICs treated with increasing bortezomib concentration. Beta-actin serves as loading control. **E**, Intracellular ¹³C-labelled β -hydroxybutyrate and ¹³C-labelled citrate levels in control or CD147 KO lung TICs cultured under ULG condition were

quantitated by LC-MS and normalized to control lung TICs (n = 9). **F**, Proliferation assay of control or CD147 KO lung TICs cultured in ULG condition supplemented with acetoacetate. Readings were determined by normalizing luminescence reading to day 0 (n = 7). **G**, Growth of control, CD147 or MCT1 KO lung TICs subcutaneously injected into flanks of NSG mice fed with standard diet (n = 4 mice). **H**, Intratumoral metabolites (non-fatty acids) and **I**, fatty acid levels in control or CD147 KO lung TICs xenografts derived from NSG mice fed with standard or ketogenic diet (n = 6 for **H** and **I**). Metabolite levels were quantitated by LC-MS and normalized to readings from control KO lung TICs. **J**, Flow cytometry profile of lung TICs xenografts excised from NSG mice fed with standard (Std) or ketogenic (Keto) diet and treated with corn oil (Veh) or AZD3965 (AZD). Cells were dissociated with excised tumors and stained with APC-conjugated CD147 antibody (n = 3). Statistical significance in **B**, **E-G**, **H-J** are determined by unpaired two tailed t-test. (* p < 0.05, **, p < 0.01, *** p < 0.001, p < 0.0001, n.s = not statistically significant).

Figure 5. CD147 could be clinically useful as a biomarker for ketone dependency in lung cancer patients. **A**, (Top) Growth of patient A003 xenograft or **B**, patient A014 xenograft subcutaneously injected into flanks of NSG mice fed with standard or ketogenic diet. (Bottom) Pictures of excised tumors are depicted below tumor growth curve (n = 4 mice). **C**, Flow cytometry profile of patient A003 or A014 xenografts excised from NSG mice fed with standard (Std) or ketogenic (Keto) diet (n = 3). Cells were dissociated with excised tumors and stained with APC-conjugated CD147 antibody. **D**, (Top) Growth of CD147⁺ and CD147⁻ lung TICs subcutaneously injected into flanks of NSG mice fed with standard diet (n = 5 mice). (Bottom) Picture of CD147⁺ tumors (in yellow circles) and CD147⁻ tumors (in red circles). **E**, Representative images from multiplex immunofluorescence staining of CD147 and DAPI on microarray slides of adenocarcinomas or squamous cell carcinomas with matched cancer adjacent lung tissue. Normal lung tissues from healthy donors were also stained on same microarray slide. Scale bar represents 100µM. **F**, Arcas of CD147⁺ staining on adenocarcinomas (Adeno) (n = 19 for tumor and cancer adjacent, n = 10 for normal) or **G**, squamous cell carcinomas (Squamous) with matched cancer adjacent lung tissue (Adj Norm) and normal lung tissues (Norm) were determined by ImageJ (n = 26 for tumor and cancer adjacent, n = 10 for normal). **H**, Box plot representing CD147 protein level of normal

tissues (n = 111) and primary tumor tissues (n = 111) from LUAD patients **I**, Kaplan-Meier survival curves comparing high and low expression of CD147 in LUAD patients (n = 1161). **J**, Box plot representing CD147 mRNAs level of treatment-naïve tissues (n = 86) and EGFR TKI-resistant tumors (n = 64) from LUAD patients. Statistical significance in **A-D** and **F-G** are determined by unpaired two tailed t-test, **H** and **J** are determined by Wilcoxon signed-rank test (* p < 0.05, **, p < 0.01, *** p < 0.001, p < 0.0001, n.s = not statistically significant).

Figure 6. Metabolic switch from glucose to ketone bodies utilization under glucose limitation promotes tumorigenesis of tumor-initiating cells. Schematic depicts how tumor-initiating cells further away from vasculature adapt to glucose limitation via metabolic switch to utilize ketone bodies from tumor microenvironment or adjacent ketogenic bulk tumor cells. As ketone bodies are more readily available than glucose in less vascularized regions of tumor, tumor-initiating cells are induced to switch from glucose to ketone bodies as a carbon source. Ketone bodies taken up via MCT1-CD147 complex are metabolized and provide energy via TCA cycle or fuel *de novo* lipogenesis. Fatty acids produced are stored as lipid droplets and could be used for membrane biogenesis or signal transduction. Collectively, this metabolic switch presents therapeutic opportunities by pharmacologically inhibiting MCT1 or FASN or inhibiting function of CD147.

Figure 7 A, Proliferation assay of lung TICs in 0, 1.4, 2.8, 5.5 or 25mM glucose. **B**, Proliferation assay of lung TICs under ULG or ULG condition supplemented with lactate. **C**, KEGG 2019 pathway enrichment analyses of upregulated genes in lung TICs cultured under ULG against HG condition or **D**, HG condition against ULG condition from RNA-seq. **E**, Heatmap shows all differentially expressed genes (FDR < 0.05) in Adh cells cultured under ultra-low glucose condition (ULG; 1.4mM) or high glucose condition (HG; 25mM). Greater variation exists between replicates rather than between comparison groups. **F**, Transcript levels of key genes in Adh cells cultured under ULG condition quantified by qPCR. These genes were previously shown to be upregulated in lung TICs cultured under ULG condition in **Fig. 1D and 1E**. Transcript levels are normalized to Adh cells cultured under HG condition. **G**, Proliferation assay of Adh cells under ULG or ULG condition supplemented with acetoacetate (n = 10 for **A**, **B**

and **G**). **H**, Circulating beta-hydroxybutyrate levels in NSG mice fed with standard or ketogenic diet. Metabolite levels were quantitated by colorimetric assay kit (n = 3). **I**, H&E staining on tumor sections excised from lung TICs xenografts or **J**, Adh cells xenografts derived from NSG mice fed with standard or ketogenic diet. Scale bar represents 50 μ M. Both proliferation assay readings in **A**, **B**, **G** were determined by normalizing luminescence reading to day 0. Statistical significance in **A-B**, **G-H** are determined by unpaired two tailed t-test. (* p < 0.05, **, p < 0.01, *** p < 0.001, p < 0.0001, n.s = not statistically significant).

Figure 8 A, Heatmap depicts all differentially detected metabolites (FDR < 0.05) from global LC-MS profiling in lung TICs cultured under ULG or ULG condition supplemented with acetoacetate as in **Fig. 2A**. Unsupervised clustering was performed. **B**, Intracellular ^{13}C -labelled β -hydroxybutyrate or **C**, ^{13}C -labelled citrate levels at 30 min and 24 hours in Adh cells cultured under ULG condition supplemented with ^{13}C -labelled β -hydroxybutyrate were quantitated by LC-MS and normalized to Adh cells cultured under ULG condition at 0 min (n = 9 for **B** and **C**). **D**, Heatmap depicts top differentially detected metabolites (FDR < 0.05) from global LC-MS profiling of tumor excised from lung TICs xenografts subcutaneously injected into flanks of NSG mice fed with standard or ketogenic diet. **E**, Intratumoral metabolites (non-fatty acids) and **F**, Fatty acid levels in Adh cells xenografts derived from NSG mice fed with standard or ketogenic diet (n = 4 for **E** and n = 5 for **F**). Metabolite levels were quantitated by LC-MS and normalized to readings from standard diet. (N.D = Not Detected).

Figure 9 A, KEGG 2019 pathway enrichment analyses of upregulated genes in lung TICs against Adh cells from RNA-seq. **B**, Two representative immunofluorescence images of BODIPY+ staining in lung TICs and Adh cells under HG condition. Scale bar represents 100 μ M. **C**, Non-polar metabolites detected using LC-MS from isolated lipid droplets of lung TICs. **D**, Representative immunofluorescence images of BODIPY+ staining in lung TICs and Adh cells under ULG condition with or without acetoacetate supplementation after 48h. **E**, Proliferation assay of replated GFP-sorted lung TICs excised and dissociated from lung TICs xenografts fed with ketogenic diet (Keto) obtained from **Fig. 3F** and cultured in-vitro in either ULG or HG condition (n = 10). **F**, Dose-response inhibition curves depict the effect of etomoxir CPT1 inhibitor on lung

TICs, Adh cells or Namec8 cells cultured in varying conditions. **G**, Oxygen consumption rate (OCR) of lung TICs under ULG condition with or without acetoacetate and in the presence or absence of palmitate ($n = 4$). Arrows depict timepoint of oligomycin, FCCP and rotenone/antimycin addition by Agilent Seahorse XFp Analyzer. **H**, Proliferation assay of lung TICs cultured in varying conditions. Basal media is derived from low glucose condition without lipoic acid, linoleic acid, Vitamin B12, choline chloride and thymidine. **I**, Western blot shows staining of embigin (EMB) and monocarboxylate transporter 2 (MCT2) involved in alternative pathway of ketone body metabolism from the whole cell lysates of lung TICs, Adh and MCF7 breast cancer cells. Beta-actin serves as loading control. Proliferation assay readings in **E**, **H** were determined by normalizing luminescence reading to day 0. Statistical significance in **E**, **H** are determined by unpaired two tailed t-test. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, $p < 0.0001$, n.s = not statistically significant).

Figure 10 A, Transcript levels of CD147 in control or CD147 KO lung TICs or **B**, Transcript levels of MCT1 in control or MCT1 KO lung TICs quantified by qPCR. Transcript levels are normalized to transcript levels of control KO lung TICs ($n = 3$ for **A** and $n = 9$ for **B**). **C**, Western blot shows staining of CD147 and MCT1 from the whole cell lysates of control KO and MCT1 KO lung TICs. **D**, Representative immunofluorescence images of MCT1 staining in control KO and CD147 KO lung TICs. White arrows depict possible MCT1 protein aggregates. Scale bar represents 100 μ M. **E**, Western blot shows staining of CD147 and MCT1 from the whole cell lysates of lung TICs and Adh under HG, ULG or ULG condition supplemented with acetoacetate. Beta-actin serves as loading control. **F**, Proliferation assay of control or CD147 KO lung TICs in HG condition ($n = 8$). Readings were determined by normalizing luminescence reading to day 0. Statistical significance in **A-B**, **F** is determined by unpaired two tailed t-test. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, $p < 0.0001$, n.s = not statistically significant). In **C**, **E** beta-actin serves as loading control.

Figure 11 A, Representative immunofluorescence images of CD147 and DAPI staining in lung cancer patient cohort in Singapore with low CD147 expression. Scale bar represents 100 μ M. **B**, Weight of NSG mice with A003 or **C**, A014 patient-derived xenograft cells subcutaneously injected into flanks as in **Fig. 5A and 5B** across duration

of in-vivo studies (n = 4 for **A** and **B**). **D**, Representative multiplex immunofluorescence images of CD147 and DAPI staining in normal lung tissues from healthy donor of microarray slides as in **Fig. 5E**. **E**, Summary of CD147^{high} (CD147 staining $50,000 < A.U^2$) and CD147^{low} (CD147 staining $< 50,000 A.U^2$) staining in adenocarcinomas or squamous cell carcinomas with matched cancer adjacent lung tissue and normal lung tissues from healthy donors. **F**, Box plot representing CD147 mRNAs level of normal tissues (n = 59) and primary tumor tissues (n = 515) from LUAD patients. **G**, Kaplan-Meier survival curves comparing high and low expression of CD147 in lung cancer patients (n = 2166). Statistical significance in **B-C** is determined by unpaired two tailed t-test, **F** is determined by Wilcoxon signed-rank test (* p < 0.05, **, p < 0.01, *** p < 0.001, p < 0.0001, n.s = not statistically significant).

Figure 12 Assessment of CD147 and CD166 staining in cancer cell line panel. Flow cytometry plots of triple-negative breast cancer (TNBC) patient-derived mammosphere BC2.2, TNBC cell lines MDA-MB-231 and SUM159, small-cell lung cancer NCI-H82, ovarian cancer cell line PA-1, gastric cancer cell line MKN-74 and mouse fibroblast cell line 3T3 stained with CD147-APC and CD166-PE.

Detailed Description

The present specification teaches methods of treating cancer. In one embodiment is a method of treating cancer or reducing the risk of recurrence of cancer in a subject, the method comprises contacting a Cluster of Differentiation 147 (CD147) overexpressing cell with an inhibitor of Monocarboxylate Transporter 1 (MCT1), fatty acid synthase (FASN) or CD147 to treat the cancer in the subject.

Disclosed herein is a method of treating cancer or reducing the risk of recurrence of cancer in a subject, the method comprises contacting a CD147 overexpressing cell with an inhibitor of MCT1 to treat the cancer in the subject.

In one embodiment, the method further comprises contacting the CD147 overexpressing cell with an inhibitor of CD147.

In one embodiment, the method further comprises contacting the CD147 overexpressing cell with an inhibitor of fatty acid synthase (FASN).

In one embodiment, there is provided a method of treating cancer or reducing the risk of recurrence of cancer in a subject, the method comprises contacting a CD147 overexpressing cell with an inhibitor of MCT1 and an inhibitor of CD147 to treat the cancer in the subject.

In one embodiment, there is provided a method of treating cancer or reducing the risk of recurrence of cancer in a subject, the method comprises contacting a CD147 overexpressing cell with an inhibitor of FASN and an inhibitor of CD147 to treat the cancer in the subject.

Without being bound by theory, the inventors have found that tumor-initiating cells (TICs) tend to reside in poorly vascularized regions of tumors and are subjected to nutrient stress. Certain TICs such as lung TICs (which overexpress CD147) are able to induce a metabolic switch from glucose towards ketone utilization specifically during glucose deprivation to drive tumorigenesis, whereas bulk, non-TIC cells are unable to do so. The inventors have shown that suppressing the ability of TICs to utilize ketones would suppress their tumorigenicity.

In one embodiment, the CD147 overexpressing cell is a tumor initiating cell. The CD147 overexpressing cell may further overexpress CD166. In one embodiment, the cell overexpresses CD147 and CD166. The CD147 overexpressing cell may further overexpress a biomarker selected from the group consisting of OXCT1, BDH1 and ACAT2. In one embodiment, the cell overexpresses CD147, CD166 and OXCT1. In one embodiment, the cell overexpresses CD147, CD166 and BDH1. In one embodiment, the cell overexpresses CD147, CD166 and ACAT2. In one embodiment, the cell overexpresses CD147, CD166, OXCT1, BDH1 and ACAT2.

The term “tumor initiating cell”, “TIC”, “cancer stem cell” or “CSC” refers to a cell that has tumor-initiating and tumor-sustaining capacity, including the ability to extensively proliferate, form new tumors and maintain cancer development, i.e., cells with indefinite

proliferative potential that drive the formation and growth of tumors. CSCs are biologically distinct from the bulk tumor cells and possess characteristics associated with stem cells, specifically the ability to self-renew and to propagate and give rise to all cell types found in a particular cancer sample. The term “cancer stem cell” or CSC includes both gene alteration in stem cells (SCs) and gene alteration in a cell which becomes a CSC.

The terms “cancer” and “cancerous” refer to or describe the physiological condition in mammals that is typically characterized in part by unregulated cell growth. As used herein, the term “cancer” refers to non-metastatic and metastatic cancers, including early stage and late stage cancers. The term “precancerous” refers to a condition or a growth that typically precedes or develops into a cancer. By “non-metastatic” is meant a cancer that is benign or that remains at the primary site and has not penetrated into the lymphatic or blood vessel system or to tissues other than the primary site. Generally, a non-metastatic cancer is any cancer that is a Stage 0, I, or II cancer, and occasionally a Stage III cancer. By “early stage cancer” is meant a cancer that is not invasive or metastatic or is classified as a Stage 0, I, or II cancer. The term “late stage cancer” generally refers to a Stage III or Stage IV cancer, but can also refer to a Stage II cancer or a sub-stage of a Stage II cancer. One skilled in the art will appreciate that the classification of a Stage II cancer as either an early stage cancer or a late stage cancer depends on the particular type of cancer. Illustrative examples of cancer include, but are not limited to, breast cancer, prostate cancer, ovarian cancer, cervical cancer, pancreatic cancer, colorectal cancer, lung cancer, hepatocellular cancer, gastric cancer, liver cancer, bladder cancer, cancer of the urinary tract, thyroid cancer, renal cancer, carcinoma, melanoma, brain cancer, non-small cell lung cancer, squamous cell cancer of the head and neck, endometrial cancer, multiple myeloma, rectal cancer, and esophageal cancer.

In one embodiment, the cancer is one that is associated with overexpression of CD147 in one or more tumor initiating cells. The cancer may be a lung cancer (such as a refractory lung cancer), a breast cancer (such as a triple-negative breast cancer) or ovarian cancer. In one embodiment, the cancer is a lung cancer (such as a refractory

lung cancer). The lung cancer may be an adenocarcinoma and/or squamous cell carcinoma.

In one embodiment, the cancer is a ketone-dependent cancer.

In one embodiment, the cancer is a metastatic cancer.

The term “recurrence” as used herein may refer to a cancer that has recurred (come back), usually after a period of time during which the cancer could not be detected. The cancer may be called a recurrent cancer. The recurrent cancer may come back to the same place as the original (primary) tumor or to another place in the body. The recurrence may be considered a “local recurrence” when the cancer is in the same place as the original cancer or very close to it. The recurrence may be a “regional recurrence” when the tumor has grown into lymph nodes or tissues near the original cancer. The recurrence may be called a distant recurrence when the cancer has spread to organs or tissues far from the original cancer. When the cancer spreads to a distant place in the body, the recurrent cancer may be called metastasis or metastatic cancer.

The “reduction” of the risk of cancer recurrence can be quantified in terms of a reduction in percentage (%) risk of cancer recurrence. For example, there can be a reduction in a risk of cancer recurrence by anywhere from about 1% to 100%. In an example, there is a reduction in risk of cancer recurrence by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% or a percentage inbetween.

The term “refractory” or “resistant” refers to a condition where there are residual cancer cells in a subject even after a complete course of treatment with a cancer therapy.

The term “relapsed” refers to a condition where a subject who was previously in remission from cancer following one or more courses of therapy re-develops cancer. A relapse may be detected by the re-appearance of cancer cells following a period of absence after completing one or more courses of treatment.

The term “subject” as used throughout the specification is to be understood to mean a human or may be a domestic or companion animal. While it is particularly contemplated that the methods of the invention are for treatment of humans, they are also applicable to veterinary treatments, including treatment of companion animals such as dogs and cats, and domestic animals such as horses, cattle and sheep, or zoo animals such as primates, felids, canids, bovids, and ungulates. The “subject” may include a person, a patient or individual, and may be of any age or gender.

The subject may be one who has been provided a ketogenic diet.

The inhibitor of MCT1, FASN or CD147 as defined herein may be a small molecule, an inhibitory nucleic acid molecule or a polypeptide.

In one embodiment, the inhibitor is a small molecule. Small molecule inhibitors that bind to the target mRNA/proteins described herein, or their binding partners, can be identified by screening of small molecule libraries. As used herein, a “small molecule” refers to a low molecular weight (< 1000 daltons, typically between ~300-700 daltons) organic compound.

The small molecule compound may be administered to a subject as a pharmaceutically acceptable salt thereof. Suitable pharmaceutically acceptable salts include, but are not limited to salts of pharmaceutically acceptable inorganic acids such as hydrochloric, sulphuric, phosphoric, nitric, carbonic, boric, sulfamic, and hydrobromic acids, or salts of pharmaceutically acceptable organic acids such as acetic, propionic, butyric, tartaric, maleic, hydroxymaleic, fumaric, maleic, citric, lactic, mucic, gluconic, benzoic, succinic, oxalic, phenylacetic, methanesulphonic, toluenesulphonic, benzenesulphonic, salicylic sulphonic, aspartic, glutamic, edetic, stearic, palmitic, oleic, lauric, pantothenic, tannic, ascorbic and valeric acids.

Base salts include, but are not limited to, those formed with pharmaceutically acceptable cations, such as sodium, potassium, lithium, calcium, magnesium, ammonium and alkylammonium. In particular, the present invention includes within its scope cationic

salts eg sodium or potassium salts, or alkyl esters (eg methyl, ethyl) of the phosphate group.

Basic nitrogen-containing groups may be quarternised with such agents as lower alkyl halide, such as methyl, ethyl, propyl, and butyl chlorides, bromides and iodides; dialkyl sulfates like dimethyl and diethyl sulfate; and others.

The small molecule compound can be administered to a subject in the form of a pharmaceutically acceptable pro-drug. The term "pro-drug" is used in its broadest sense and encompasses those derivatives that are converted *in vivo* to the compound of the invention. Such derivatives would readily occur to those skilled in the art.

The compound may be in crystalline form either as the free compound or as a solvate (e.g. hydrate) and it is intended that both forms are within the scope of the present invention. Methods of solvation are generally known within the art.

In other embodiments, the inhibitor is a peptide or polypeptide. This includes peptide aptamers, thioredoxins, monobodies, anticalin, Kunitz domains, avimers, knottins, fynomers, atrimers, DARPins, affibodies, nanobodies (i.e. single-domain antibodies (sdAbs)) affilins, armadillo repeat proteins (ArmRPs), OBodies and fibronectin – reviewed e.g. in Reverdatto et al., *Curr Top Med Chem.* 2015; 15(12): 1082–1101, which is hereby incorporated by reference in its entirety (see also e.g. Boersma et al., *J Biol Chem* (2011) 286:41273-85 and Emanuel et al., *Mabs* (2011) 3:38-48). Inhibitors include peptides/polypeptides that can be identified by screening of libraries of the relevant peptides/polypeptides. The peptide/polypeptide inhibitors may be referred to as inhibitory peptides/polypeptides.

In one embodiment, the inhibitor is an inhibitor of MCT1.

The major chemical classes of MCT1 inhibitors include Cyanoacetic Acid Derivates, Coumarin Derivatives, Flavone Derivates, Indole Cyanoacrylic Acids, Uracil Derivatives, Pyrazole Derivatives, and Chromenone Derivatives.

In some embodiments, the MCT1 inhibitor is a compound of formula (1) as disclosed in WO2004065394, which is incorporated by reference in its entirety for the teaching of these MCT1 inhibitors.

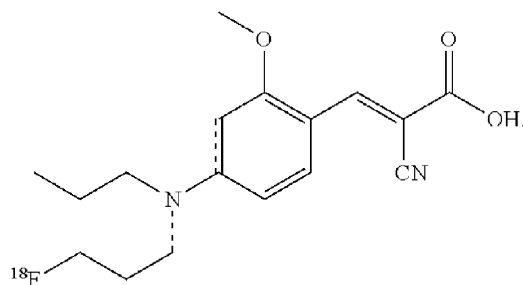
In some embodiments, the MCT1 inhibitor is a compound of formula (A), (B), or (C) as disclosed in PCT Published Patent Application WO2016118825, which is incorporated by reference in its entirety for the teaching of these MCT1 inhibitors

In some embodiments, the MCT inhibitor is AZD3965 (CAS Number: 1448671-31-5).

In some embodiments, the MCT inhibitor is AR-C155858 (CAS Number: 496791-37-8).

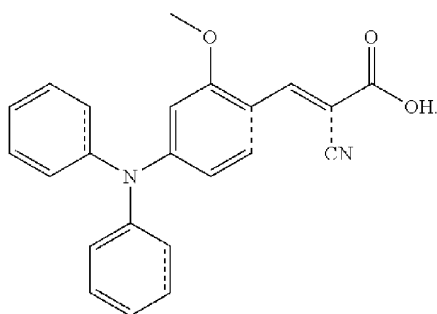
In some embodiments, the MCT inhibitor is α -cyano-4-hydroxycinnamic (α -CCA) (CAS Number: 28166-41-8).

In some embodiments, the MCT inhibitor is FACH or [F18]FACH, having the formula:



In some embodiments, the MCT is compound A, having the formula:

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In one embodiment, the inhibitor of MCT1 is AZD3965, BAY-8002, 7ACC2 or an analogue thereof.

Inhibitors of FASN include the following molecules: 1,3-dibromopropanone, Ellman's reagent 5,5'-dithiobis(2-nitrobenzoic acid), DTNB, 4-(4'-chlorobenzoyloxy) benzyl nicotinate (KCD-232), 4-(4'-chlorobenzoyloxy) benzoic acid (MII), 2-[5(4-chlorophenyl)pentyl]oxirane-2-carboxylate (POCA) and its CoA derivative, ethoxyformic anhydride, thiolactomycin, cerulenin, phenyocerulenin, melarsoprol, iodoacetate, phenylarsineoxide, pentostam, melittin or methyl malonyl CoA.

In one embodiment, the inhibitor of FASN is cerulenin, or an analogue thereof, cerulenin derivative C75 or orlistat.

In one embodiment, the inhibitor of CD147 is an antigen-binding molecule.

By "antigen-binding molecule" is meant a molecule that has binding affinity for a target antigen. It will be understood that this term extends to immunoglobulins, immunoglobulin fragments and non-immunoglobulin derived protein frameworks that exhibit antigen-binding activity. Representative antigen-binding molecules that are useful in the practice of the present invention include antibodies and their antigen-binding fragments. The term "antigen-binding molecule" includes antibodies and antigen-binding fragments of antibodies.

Antigen-binding molecules can be naked or conjugated to other molecules or moieties such as toxins, radioisotopes, small molecule drugs, polypeptides, etc.

The term "antibody molecule" or "antibody" refers to a protein, e.g., an immunoglobulin chain or fragment thereof, comprising at least one immunoglobulin variable domain sequence. The term "antibody molecule" includes, for example, a monoclonal antibody (including a full length antibody which has an immunoglobulin Fc region). An antibody molecule comprises a full length antibody, or a full length immunoglobulin chain, or an antigen binding or functional fragment of a full length antibody, or a full length immunoglobulin chain. An antibody molecule can also be a multi-specific antibody molecule, e.g., it comprises a plurality of immunoglobulin variable domain sequences, wherein a first immunoglobulin variable domain sequence of the plurality has binding specificity for a first epitope and a second immunoglobulin variable domain sequence of the plurality has binding specificity for a second epitope.

An antibody molecule can be derivatized or linked to another functional molecule (e.g., another peptide or protein). Methods of derivatization include but are not limited to the addition of a fluorescent moiety, a radionucleotide, a toxin, an enzyme or an affinity ligand such as biotin. Accordingly, the antibody molecules of the invention are intended to include derivatized and otherwise modified forms of the antibodies described herein, including immunoadhesion molecules. For example, an antibody molecule can be functionally linked (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (e.g., a bispecific antibody or a diabody), a detectable agent, a cytotoxic agent, a pharmaceutical agent, and/or a protein or peptide that can mediate association of the antibody or antibody portion with another molecule (such as a streptavidin core region or a poly-histidine tag).

By "binding fragment" or "antigen-binding fragment" of an antibody, it is intended to indicate any peptide, polypeptide, or protein retaining the ability to bind to the target (also generally referred as antigen) of the antibody. In an embodiment, such "antigen binding fragments" are selected in the group consisting of Fv, scFv (sc for single chain), Fab, F(ab')₂, Fab', scFv-Fc fragments or diabodies, or any fragment of which the half-

life time would have been increased by chemical modification, such as the addition of poly(alkylene) glycol such as poly(ethylene) glycol ("PEGylation") (pegylated fragments called Fv-PEG, scFv-PEG, Fab-PEG, F(ab')₂-PEG or Fab'-PEG) ("PEG" for Poly(Ethylene) Glycol), or by incorporation into a liposome. In one embodiment, the "antigen binding fragments" will be constituted or will comprise a partial sequence of the heavy or light variable chain of the antibody from which they are derived, said partial sequence being sufficient to retain the same specificity of binding as the antibody from which it is descended and a sufficient affinity, for example at least equal to 1/100, as a further example to at least equal to 1/10, of the affinity of the antibody from which it is descended, with respect to the target.

Examples of CD147 inhibitors include Metuzumab, AC-73, CD147 antagonist peptide-9.

In some embodiments, the inhibitor comprises an inhibitory nucleic acid molecule. Examples of inhibitory nucleic acid molecules include, but are not limited to, antisense nucleic acid molecules, small interfering RNAs (siRNAs), and short hairpin RNAs (shRNAs), or a nucleic acid encoding the same. Such inhibitory nucleic acid molecules can be designed to target any region of a MCT1, FASN or CD147 nucleic acid molecule, such as an mRNA molecule. In some embodiments, the antisense RNA, siRNA, or shRNA hybridizes to a sequence within a MCT1, FASN or CD147 genomic nucleic acid molecule or mRNA molecule and decreases expression of a MCT1, FASN or CD147 polypeptide in a cell in the subject. In some embodiments, the inhibitor comprises an antisense RNA that hybridizes to a MCT1, FASN or CD147 genomic nucleic acid molecule or mRNA molecule and decreases expression of the MCT1, FASN or CD147 polypeptide in a cell in the subject. In some embodiments, the inhibitor comprises an siRNA that hybridizes to a MCT1, FASN or CD147 genomic nucleic acid molecule or mRNA molecule and decreases expression of the MCT1, FASN or CD147 polypeptide in a cell in the subject. In some embodiments, the inhibitor comprises an shRNA that hybridizes to a MCT1, FASN or CD147 genomic nucleic acid molecule or mRNA molecule and decreases expression of the MCT1, FASN or CD147 polypeptide in a cell in the subject.

As used herein, the terms “nucleic acid”, “nucleic acid molecule”, “nucleic acid sequence”, “polynucleotide”, or “oligonucleotide” can comprise a polymeric form of nucleotides of any length, can comprise DNA and/or RNA, and can be single-stranded, double-stranded, or multiple stranded. One strand of a nucleic acid also refers to its complement.

Provided herein is a pharmaceutical composition comprising an inhibitor as defined herein.

The term “pharmaceutical composition” or “pharmaceutical formulation” refers to a preparation which is in such form as to permit the biological activity of the active ingredient(s) to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the composition or formulation would be administered. Such formulations are sterile. “Pharmaceutically acceptable” carriers and excipients (vehicles, additives) are those which can reasonably be administered to a subject mammal to provide an effective dose of the active ingredient employed.

In one embodiment, inhibiting the tumor initiating cell inhibits tumor growth in the subject.

Provided herein is a pharmaceutical combination comprising an inhibitor of MCT1 and an inhibitor of CD147.

Also provided herein is a pharmaceutical combination comprising an inhibitor of FASN and an inhibitor of CD147.

Also provided herein is a pharmaceutical combination comprising an inhibitor of MCT1 and an inhibitor of FASN.

In the present disclosure the term “pharmaceutical combination” refers to a non-fixed combination. The term “non-fixed combination” means that the active ingredients, e.g. an inhibitor of MCT1 and an inhibitor of FASN are both administered to a patient as separate entities either simultaneously or sequentially with no specific time limits,

wherein such administration provides therapeutically effective levels of the two compounds in the body of the patient.

The terms "a combination" or "in combination with," it is not intended to imply that the therapy or the therapeutic agents must be administered at the same time and/or formulated for delivery together, although these methods of delivery are within the scope described herein. The therapeutic agents in the combination can be administered concurrently with, prior to, or subsequent to, one or more other additional therapies or therapeutic agents. The therapeutic agents or therapeutic protocol can be administered in any order. In general, each agent will be administered at a dose and/or on a time schedule determined for that agent. It will further be appreciated that the additional therapeutic agent utilized in this combination may be administered together or separately in different compositions. In general, it is expected that additional therapeutic agents utilized in combination be utilized at levels that do not exceed the levels at which they are utilized individually. In some embodiments, the levels utilized in combination will be lower than those utilized individually.

Provided herein is a method of treating cancer or reducing the risk of recurrence of cancer in a subject, wherein the method comprises inhibiting a CD147 overexpressing cell with an inhibitor of MCT1, FASN or CD147 to treat the cancer or reduce the risk of recurrence of cancer in the subject.

Disclosed herein is a method of treating cancer or reducing the risk of recurrence of cancer in a subject, wherein the method comprises inhibiting a CD147 overexpressing cell with an inhibitor of MCT1 to treat the cancer or reduce the risk of recurrence of cancer in the subject.

In one embodiment, the method further comprises contacting the CD147 overexpressing cell with an inhibitor of CD147.

The term "treating" as used herein may refer to (1) preventing or delaying the appearance of one or more symptoms of the disorder; (2) inhibiting the development of the disorder or one or more symptoms of the disorder; (3) relieving the disorder, i.e.,

causing regression of the disorder or at least one or more symptoms of the disorder; and/or (4) causing a decrease in the severity of one or more symptoms of the disorder.

Provided herein is a method of treating cancer or reducing the risk of recurrence of cancer in a subject, wherein the method comprises administering to the subject an inhibitor of MCT1, FASN or CD147 to treat the cancer or reduce the risk of recurrence of cancer in the subject, wherein the cancer is associated with overexpression of CD147 in one or more tumor initiating cells.

Disclosed herein is a method of treating cancer or reducing the risk of recurrence of cancer in a subject, wherein the method comprises administering to the subject an inhibitor of MCT1 to treat the cancer or reduce the risk of recurrence of cancer in the subject, wherein the cancer is associated with overexpression of CD147 in one or more tumor initiating cells.

The term “administering” refers to contacting, applying or providing an inhibitor of MCT1, FASN or CD147 to a subject.

The method as defined herein may comprise administering an effective amount of the inhibitor to the subject.

By “effective amount,” in the context of treating or preventing a disease or condition (e.g., a cancer) is meant the administration of an amount of active agent to a subject, either in a single dose or as part of a series or slow release system, which is effective for the treatment or prevention of that disease or condition. The effective amount will vary depending upon the health and physical condition of the subject and the taxonomic group of individual to be treated, the formulation of the composition, the assessment of the medical situation, and other relevant factors.

Provided herein is an inhibitor of MCT1, FASN or CD147 for use in treating cancer or reducing the risk of recurrence of cancer in the subject, wherein the cancer is associated with overexpression of CD147 in one or more tumor initiating cells.

Disclosed herein is an inhibitor of MCT1 for use in treating cancer or reducing the risk of recurrence of cancer in the subject, wherein the cancer is associated with overexpression of CD147 in one or more tumor initiating cells. In one embodiment, the inhibitor of MCT1 is to be administered with an inhibitor of CD147 to the subject.

Provided herein is the use of an inhibitor of MCT1, FASN or CD147 in the manufacture of a medicament for treating cancer or reducing the risk of recurrence of cancer in the subject, wherein the cancer is associated with overexpression of CD147 in one or more tumor initiating cells.

Disclosed herein is the use of an inhibitor of MCT1 in the manufacture of a medicament for treating cancer or reducing the risk of recurrence of cancer in the subject, wherein the cancer is associated with overexpression of CD147 in one or more tumor initiating cells. In one embodiment, the medicament is to be administered with an inhibitor of CD147 to the subject.

Dosing may occur at intervals of minutes, hours, days, weeks, months or years or continuously over any one of these periods. Suitable dosages may lie within the range of about 0.1 ng per kg of body weight to 1 g per kg of body weight per dosage, such as is in the range of 1 mg to 1 g per kg of body weight per dosage. In one embodiment, the dosage may be in the range of 1 mg to 500 mg per kg of body weight per dosage. In another embodiment, the dosage may be in the range of 1 mg to 250 mg per kg of body weight per dosage. In yet another embodiment, the dosage may be in the range of 1 mg to 100 mg per kg of body weight per dosage, such as up to 50 mg per body weight per dosage.

Suitable dosage amounts and dosing regimens can be determined by the attending physician and may depend on the severity of the condition as well as the general age, health and weight of the patient to be treated.

The inhibitors as defined herein may be administered in a single dose or a series of doses. While it is possible for the active ingredient to be administered alone, it is preferable to present it as a composition, preferably as a pharmaceutical composition.

The formulation of such compositions is well known to those skilled in the art. The composition may contain any suitable carriers, diluents or excipients. These include all conventional solvents, dispersion media, fillers, solid carriers, coatings, antifungal and antibacterial agents, dermal penetration agents, surfactants, isotonic and absorption agents and the like. It will be understood that the inhibitors as defined herein may also include other supplementary physiologically active agents.

The carrier must be pharmaceutically "acceptable" in the sense of being compatible with the other ingredients of the composition and not injurious to the patient. The compositions may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

Injectables for such use can be prepared in conventional forms, either as a liquid solution or suspension or in a solid form suitable for preparation as a solution or suspension in a liquid prior to injection, or as an emulsion. Carriers can include, for example, water, saline (e.g., normal saline (NS), phosphate-buffered saline (PBS), balanced saline solution (BSS)), sodium lactate Ringer's solution, dextrose, glycerol, ethanol, and the like; and if desired, minor amounts of auxiliary substances, such as wetting or emulsifying agents, buffers, and the like can be added. Proper fluidity can be maintained, for example, by using a coating such as lecithin, by maintaining the required particle size in the case of dispersion and by using surfactants.

Other modes of administration including topical or intravenous administration may also be possible. For example, solutions or suspensions of the inhibitors may be formulated. Topical application typically involves administering the inhibitors in an amount between 0.1 ng and 10 mg. The inhibitors may also be suitable for intravenous administration.

The inhibitors as defined herein may also be suitable for oral administration and may be presented as discrete units such as capsules, sachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste. In another embodiment, the inhibitors as defined herein are orally administrable.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (e.g. inert diluent, preservative disintegrant (e.g. sodium starch glycolate, cross-linked polyvinyl pyrrolidone, cross-linked sodium carboxymethyl cellulose) surface-active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

The inhibitors may be suitable for topical administration in the mouth including lozenges comprising the active ingredient in a flavoured base, usually sucrose and acacia or tragacanth gum; pastilles comprising the active ingredient in an inert basis such as gelatine and glycerin, or sucrose and acacia gum; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

The inhibitors may be suitable for topical administration to the skin may comprise the compounds dissolved or suspended in any suitable carrier or base and may be in the form of lotions, gel, creams, pastes, ointments and the like. Suitable carriers include mineral oil, propylene glycol, polyoxyethylene, polyoxypropylene, emulsifying wax, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetaryl alcohol, 2-

octyldodecanol, benzyl alcohol and water. Transdermal patches may also be used to administer the compounds of the invention.

The inhibitors may be suitable for parenteral administration include aqueous and non-aqueous isotonic sterile injection solutions which may contain anti-oxidants, buffers, bactericides and solutes which render the inhibitors isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The inhibitors may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Preferred unit dosage composition are those containing a daily dose or unit, daily sub-dose, as herein above described, or an appropriate fraction thereof, of the active ingredient.

It should be understood that in addition to the active ingredients particularly mentioned above, the composition of this invention may include other agents conventional in the art having regard to the type of composition in question, for example, those suitable for oral administration may include such further agents as binders, sweeteners, thickeners, flavouring agents disintegrating agents, coating agents, preservatives, lubricants and/or time delay agents. Suitable sweeteners include sucrose, lactose, glucose, aspartame or saccharine. Suitable disintegrating agents include cornstarch, methylcellulose, polyvinylpyrrolidone, xanthan gum, bentonite, alginic acid or agar. Suitable flavouring agents include peppermint oil, oil of wintergreen, cherry, orange or raspberry flavouring. Suitable coating agents include polymers or copolymers of acrylic acid and/or methacrylic acid and/or their esters, waxes, fatty alcohols, zein, shellac or gluten. Suitable preservatives include sodium benzoate, vitamin E, alpha-tocopherol, ascorbic acid, methyl paraben, propyl paraben or sodium bisulphite. Suitable lubricants include magnesium stearate, stearic acid, sodium oleate, sodium chloride or talc. Suitable time delay agents include glyceryl monostearate or glyceryl distearate.

Disclosed herein is a method of detecting a cancer that is likely to be susceptible to treatment with an inhibitor of MCT1, FASN or CD147, the method comprising detecting increased level of expression of CD147 in a sample obtained from the subject, wherein an increased level of expression of CD147 as compared to a reference identifies the subject as one who is suitable for treatment with an inhibitor of ketone metabolism.

In one embodiment, the method comprises detecting increase level of expression of CD147 in one or more cells from a sample obtained from the subject.

In one embodiment, the one or more cells are cancer cells. The one or more cancer cells may be tumor initiating cells.

The term “sample” herein is used in its broadest sense. In one sense, it is meant to include a specimen or culture obtained from any source, including both biological and environmental sources. A “biological sample” includes within its scope a collection of similar fluids, cells, or tissues isolated from a biological source, such as a whole organism or in vitro culture. Samples include but are not limited to tissue biopsies, tissue resections, tissue aspirates, swabs (e.g., buccal swabs), whole blood, plasma, serum, urine, saliva, cerebrospinal fluid, and cell cultures, and may be obtained using any suitable method known in the art. Archival tissues, such as those having treatment or outcome history may also be used for sample extraction. The sample may be pooled from multiple aliquots. Samples include untreated, treated, diluted and concentrated samples.

A “biological fluid” herein includes, but is not limited to, intravascular fluid (e.g., blood, plasma, serum, lymph), urine, saliva, sputum, cerebrospinal fluid, pleural fluid, fluid of the respiratory, intestinal, and genitourinary tracts, synovial fluid, vaginal secretion, tear fluid, pus, breast milk, semen, fluid from ascites, cyst or tumour, amniotic fluid, or combinations thereof.

Samples as referred to herein may be tissue samples, biological fluids, or cultures derived from tissue or fluid samples. In one embodiment, the sample is a bodily fluid or a liquid biopsy. Bodily fluids include but are not limited to peripheral blood, serum,

plasma, ascites, urine, cerebrospinal fluid (CSF), sputum, saliva, bone marrow, synovial fluid, aqueous humor, amniotic fluid, cerumen, breast milk, bronchoalveolar lavage fluid, semen (including prostatic fluid), Cowper's fluid or pre-ejaculatory fluid, female ejaculate, sweat, fecal matter, tears, cyst fluid, pleural fluid, peritoneal fluid, pericardial fluid, lymph, chyme, chyle, bile, interstitial fluid, menses, pus, sebum, vomit, vaginal secretions, mucosal secretion, stool water, pancreatic juice, lavage fluids from sinus cavities, bronchopulmonary aspirates and other lavage fluids. In one embodiment, the sample is a blood, serum or plasma sample.

In some embodiments, the sample is a cancer sample. As used herein, a "cancer sample" refers to a sample derived from a cancerous cell or tissue. Cancer samples would typically contain cancer cells, circulating tumor cells (CTCs), and/or cellular material (e.g., cellular vesicles, secretions or debris) derived from cancer cells or CTCs.

Samples may be treated prior to detection of a biomarker. For example, cellular fractions, nucleic acid fractions or protein fractions may be extracted for analysis. Extraction of nucleic acids and proteins may be achieved with various methods known in the art. For example, RNA extraction may be achieved using protein precipitation according to standard procedures and techniques known in the art. Such methods may utilise a nucleic acid-binding column to capture nucleic acids contained within the extracellular vesicles. Once bound, the nucleic acids can then be eluted using a buffer or solution suitable to disrupt the interaction between the nucleic acids and the binding column, thereby eluting the nucleic acids. Alternatively, commercially available kits for purifying proteins and RNA may be used.

The reference as referred to herein may be the expression level of a biomarker in a sample from a subject of the same species without cancer, or an average expression level in samples from a population of subjects of the same species (e.g., of varying ages, ethnic backgrounds and genders) without cancer. The reference may be the expression level of a biomarker in a non-cancerous tissue sample from the same subject. The reference may also be the expression level in a sample from the same subject before the suspected onset of a cancer. Reference values may be mRNA or protein levels. The reference values can be a pre-determined value, and can be stored in a database and used

as a reference in subsequent analyses. In one embodiment, methods herein comprise the step of comparing the level of expression of a biomarker in the sample to the reference.

The measured expression level of a gene may first be normalised before comparison with a reference. Normalisation is typically used to control for unwanted biological variation. In a non-limiting example, biological variation can result from some feature of the subject or the sample collection that is not relevant to the methods of the present disclosure, such as variations created by collecting samples at different times of the day and variations due to the age or gender of the subject.

Normalisation can be performed using methods known in the art. In a non-limiting example, normalisation is performed by dividing the measured expression level of a biomarker by the expression level of a reference or housekeeping gene. Useful reference or housekeeping genes are genes that show a low variation in their expression level across a variety of different samples and subjects. For example, a useful reference gene will show the same expression level in samples derived from subjects who have cancer and in samples derived from subjects who do not have cancer. Such reference or housekeeping genes are typically genes which are crucial for fundamental cellular processes such as metabolism and cell structure maintenance.

As used herein, the term “increase” or “increased” with reference to a biomarker refers to a statistically significant and measurable increase in the biomarker as compared to a reference. The increase may be an increase of at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 100%.

As used herein, the term “decrease” or “decreased” with reference to a biomarker refers to a statistically significant and measurable decrease in the biomarker as compared to a reference. The decrease may be a decrease of at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90%.

In one embodiment, an increase in the level of a biomarker as compared to a reference is an increase of 1.1 fold, 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 1.6 fold, 1.7 fold, 1.8 fold, 1.9 fold, 2 fold, 3 fold, 4 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, 10 fold, 11 fold, 12 fold, 13 fold, 14 fold, 15 fold, 16 fold, 17 fold, 18 fold, 19 fold, 20 fold, 21 fold, 22 fold, 23 fold, 24 fold, 25 fold, 26 fold, 27 fold, 28 fold, 29 fold, 30 fold, 31 fold, 32 fold, 33 fold, 34 fold, 35 fold, 36 fold, 37 fold, 38 fold, 39 fold, 40 fold, 41 fold, 42 fold, 43 fold, 44 fold, 45 fold, 46 fold, 47 fold, 48 fold, 49 fold, 50 fold, 51 fold, 52 fold, 53 fold, 54 fold, 55 fold, 56 fold, 57 fold, 58 fold, 59 fold, 60 fold, 61 fold, 62 fold, 63 fold, 64 fold, 65 fold, 66 fold, 67 fold, 68 fold, 69 fold, 70 fold, 71 fold, 72 fold, 73 fold, 74 fold, 75 fold, 76 fold, 77 fold, 78 fold, 79 fold, 80 fold, 81 fold, 82 fold, 83 fold, 84 fold, 85 fold, 86 fold, 87 fold, 88 fold, 89 fold, 90 fold, 91 fold, 92 fold, 93 fold, 94 fold, 95 fold, 96 fold, 97 fold, 98 fold, 99 fold or 100 fold increase, or anywhere in between.

Methods herein may be combined with other biomarkers or other clinical methods to provide a more comprehensive prediction of treatment outcome. Such biomarkers may include non-genetic factors such as demographic or clinical variables, non-limiting examples of which include gender, age, lifestyle (e.g., diet, frequency of physical activity, alcohol intake, tobacco use), physiological parameters (e.g., body mass index, blood pressure), family history of disease, clinical history of cancer and/or other comorbid diseases or conditions, and other diagnostic indications of cancer (e.g., levels of other biomarkers, medical imaging). Clinical methods may include imaging methods and histopathological analyses of biopsy samples.

Methods herein may be performed prior to, during or after the cancer therapy, and may be performed on multiple occasions over a period of time.

Provided herein is a method of treating cancer or reduce the risk of recurrence in a subject, the method comprising: a) detecting increased level of expression of CD147 in a sample obtained from the subject, wherein an increased level of expression of CD147 as compared to a reference identifies the subject as one who is suitable for treatment with an inhibitor of MCT1, FASN or CD147, and b) administering an inhibitor of MCT1, FASN or CD147 to the subject so as to treat the cancer or reduce the risk of recurrence of cancer in the subject.

Disclosed herein is a method of treating cancer or reduce the risk of recurrence in a subject, the method comprising: a) detecting increased level of expression of CD147 in a sample obtained from the subject, wherein an increased level of expression of CD147 as compared to a reference identifies the subject as one who is suitable for treatment with an inhibitor of MCT1, FASN or CD147, and b) administering an inhibitor of MCT1 to the subject so as to treat the cancer or reduce the risk of recurrence of cancer in the subject. The method may further comprise administering to the subject an inhibitor of CD147.

The method may further comprise detecting increased level of expression of CD147 and CD166 in a sample obtained from the subject, wherein an increased level of expression of CD147 and CD166 as compared to a reference identifies the subject as one who is suitable for treatment with an inhibitor of MCT1, FASN or CD147.

Provided herein is a method of treating cancer or reduce the risk of recurrence in a subject, the method comprising: a) selecting a subject having increased level of expression of CD147 in a sample obtained from the subject, wherein an increased level of expression of CD147 as compared to a reference identifies the subject as one who is suitable for treatment with an inhibitor of MCT1, FASN or CD147, and b) administering an inhibitor of MCT1, FASN or CD147 to the subject so as to treat the cancer or reduce the risk of recurrence of cancer in the subject.

Disclosed herein is a method of treating cancer or reduce the risk of recurrence in a subject, the method comprising: a) selecting a subject having increased level of expression of CD147 in a sample obtained from the subject, wherein an increased level of expression of CD147 as compared to a reference identifies the subject as one who is suitable for treatment with an inhibitor of MCT1, FASN or CD147, and b) administering an inhibitor of MCT1 to the subject so as to treat the cancer or reduce the risk of recurrence of cancer in the subject. The method may further comprise administering to the subject an inhibitor of CD147.

Disclosed herein is a method of stratifying a subject into one who is likely to be suffering from an aggressive or a non-aggressive cancer, the method comprising analyzing the level of CD147 expression in a sample that is obtained from the subject, wherein i) an increased level of expression of CD147 as compared to a reference identifies the subject as one who is likely to be suffering from an aggressive cancer and ii) a lack of increase of expression of CD147 identifies the subject as one who is likely to be suffering from a non-aggressive cancer.

Disclosed herein is a method of predicting a likelihood of a subject who is suffering from an aggressive cancer, wherein the method comprises detecting increased level of expression of CD147 in a sample obtained from the subject, wherein an increased level of expression of CD147 in the sample as compared to a reference indicates that the subject is likely to be suffering from an aggressive cancer.

As used herein, “and/or” refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative (or).

As used in this application, the singular form “a,” “an,” and “the” include plural references unless the context clearly dictates otherwise. For example, the term “an agent” includes a plurality of agents, including mixtures thereof.

Throughout this specification and the statements which follow, unless the context requires otherwise, the word “comprise”, and variations such as “comprises” and “comprising”, will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications which fall within the spirit and scope. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

Unless otherwise defined, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs.

Certain embodiments of the invention will now be described with reference to the following examples which are intended for the purpose of illustration only and are not intended to limit the scope of the generality hereinbefore described.

EXAMPLES

METHODS

Table 1: KEY RESOURCES TABLE

REAGENTS OR RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-GAPDH	Santa Cruz	Cat#sc-47724; RRID:AB_627678
Anti- β -Actin	Santa Cruz	Cat#sc-47778; RRID:AB_626632
Anti-MCT1 (H-1)	Santa Cruz	Cat#sc-365501; RRID:AB_10841766
Anti-MCT2 (D-5)	Santa Cruz	Cat#sc-166925; RRID:AB_10607820
Anti-PPAR α (H-2)	Santa Cruz	Cat#sc-398394; RRID:AB_2885073
Anti-PGC1 α (D-5)	Santa Cruz	Cat#sc-518025; RRID:AB_2890187
Anti-FASN (A-5)	Santa Cruz	Cat#sc-55580; RRID:AB_2231427
Anti-CPT1	Santa Cruz	Cat#sc-393070

Anti-OXCT1	Abcam	Cat#ab7041; RRID:AB_1310527
Anti-ACAT1 [9H10AB4]	Abcam	Cat#ab110290; RRID:AB_10864779
Anti-ACAT2	Abcam	Cat#ab74901; RRID:AB_2049131
Anti-CD147 [EPR4053]	Abcam	Cat#ab108308; RRID:AB_10861230
Anti-Embigin	Abcam	Cat#ab127692; RRID:AB_11129985
Anti-CD147 (8D12), APC	Thermo Fisher Scientific	Cat#17-1472-42; RRIAD: AB_10714990

Chemicals and cell lines

Patient-derived NSCLC lung TICs cell line was derived from NSCLC tumors collected as described previously. In brief, tumor samples were dissociated with collagenase/dispase mixture in a 1:1 ratio at 1mg/mL. Cell clusters and red blood cells were removed by lysing of red blood cells and filtering through Falcon® 40 µm cell strainer. Cells were then resuspended and cultured in Dulbecco's modified Eagle's medium F-12 with HEPES (DMEM F-12; Gibco, Life Technologies, Vienna, Austria). The culture media is supplemented with 4mg/mL BSA, 20ng/mL epidermal growth factor (EGF), 4ng/mL basic fibroblast growth factor (bFGF) and insulin-transferrin selenium (ITS), all purchased from Sigma Aldrich, Vienna, Austria. TICs were then maintained by sorting for CD166 expression at regular intervals and prior to transplantation experiments. Patient-derived NSCLC Adh cell line was cultured in Dulbecco's modified Eagle's medium F-12 with HEPES (DMEM F-12; Gibco, Life Technologies, Vienna, Austria) supplemented with 10% (v/v) fetal bovine serum (FBS). 293 cell line used for transfection experiments was cultured in tissue culture dishes with Dulbecco's Modified Eagle Medium (DMEM) media supplemented with 10% (v/v) FBS. Penicillin-Streptomycin, L-glutamine, Minimum Essential Medium non-essential amino acids (MEM NEAA) and sodium pyruvate (all Gibco, Life Technologies, Vienna, Austria) were also supplemented if necessary. Naturally Arising MEsenchymal Cells (NAMEC8), which are derived from human mammary epithelial cells (HMLE), were cultured in Mammary Epithelial Cell Growth Medium (MEGM) supplemented with bovine pituitary extract (BPE), human epidermal growth factor (hEGF), insulin, hydrocortisone and gentamicin sulfate-Amphotericin (GA-1000). In brief, NAMEC8 was isolated from HMLE cells by differential trypsinization for one minute with 0.05%

trypsin, selecting for cells with mesenchymal phenotype that could be passaged. Lithium acetoacetate (sc-215253A), 3-hydroxybutyrate (sc-231749), D-(+)-Glucose (G7021) and sodium L-lactate (L7022) were all purchased from Sigma Aldrich, Vienna, Austria.

Metabolomic analyses

Isotope-labelled [^{13}C] sodium D-3-hydroxybutyrate was purchased from Cambridge Isotope Laboratories (Massachusetts, United States of America). LC-MS grade methanol and ammonium bicarbonate were obtained from Fisher Chemical (Hampton, NH). Ultra-high- quality water from an Atrium® Pro lab water system (Sartorius, Gottingen, Germany) was used as the water source throughout this study. LC-MS grade acetonitrile and chloroform were purchased from Merck (Darmstadt, Germany). LC-MS grade formic acid (FA) and ammonia solution (25%) were from VWR Chemical (Singapore). Tricine and sodium chloride were obtained from Sigma Aldrich (Tokyo, Japan).

Cell pellets were extracted using a two-phase liquid-liquid extraction protocol. Methanol, chloroform, and 3.8 mM tricine solution (approximately 1:1:0.5 (v/v)) were used to separate lipid species (organic top fraction) from polar metabolites. The organic fraction which comprised mainly of chloroform were collected in 2 mL amber glass vial (Sigma Aldrich) and the headspace was purged with nitrogen to minimize sample degradation before storing at $-80\text{ }^{\circ}\text{C}$. Prior to LC-MS of acyl-CoA analysis, the lipid extracts were dried down under a stream of nitrogen gas and reconstituted in 1:1 (v/v) ratio of buffer A and buffer B of the following LC-MS analysis for lipid metabolomics. In the analysis of hydroxybutyrate, the aqueous fraction was collected in 2 mL microcentrifuge tube (Eppendorf, Hamburg, Germany). All extracts were stored at $-80\text{ }^{\circ}\text{C}$ before LC-MS analysis. The aqueous extracts were dried down at $4\text{ }^{\circ}\text{C}$ under vacuum pressure using a CentriVap centrifugal vacuum concentrator (Labconco, Kansas, MO) and reconstituted with 5% (v/v) methanol in water solution before LC-MS analysis.

Liquid chromatography-mass spectrometry (LC-MS) and data analysis

LC-MS and LC-MSMS analyses were performed using an ACQUITY LC (Waters, Milford, MA) interfaced with TripleTOF® 6600 MS (Sciex, Framingham, MA) via a OptiFlow™ Interface (duo spray ion source) where the ion source was heated to $500\text{ }^{\circ}\text{C}$

under the control of Analyst® TF Software (version 1.7.1, Sciex). For the positive mode in the mass range of 100 to 1800 m/z with 100 ms cycle time and 50 to 1,200 m/z in the negative mode. Calibrant delivery system (CDS) was used to introduce calibration solution for automated mass calibration of the mass spectrometer, to ensure that the mass accuracy of the system is maintained throughout batch acquisition. The calibration compound was Reserpine (m/z 609.28066) for positive mode and Sulfinpyrazone (m/z 403.11219) for negative mode. The MS acquisition parameters were set at the following: Ion source voltage was set to 5500 V for positive mode and 3000 V for negative mode, nebulizer gases (GS1 and GS2) 50 psi, curtain gas (CUR) 40 psi, source temperature (TEM) 500°C, declustering potential (DP) 80 V, collision energy (CE) 5 V and collision energy spread (CES) 20 V.

An ACQUITY BEH (C18) column (2.1 mm × 100.0 mm, 1.7 µm, Waters) was used and chromatography was conducted in gradient elution mode at flow rate of 0.4 mL/min. The LC mobile phase composition are water with 20mM ammonium bicarbonate and 0.1% (v/v) ammonia solution for solvent A, acetonitrile with 0.1% (v/v) ammonia solution for solvent B. Acyl-CoA species were separated using the following gradient condition: 0-0.5 min at 5% B, ramping from 5-50% B during 0.5-1 min, ramping from 50-80% B during 1-4 min; increase gradient from 80% to 100% during 4-4.2min before maintaining at 100% B from 4.2-6 min. Injection volume of samples was 2 µL.

For hydroxybutyrate analysis, all chromatographic separations were performed as described previously. Briefly, polar metabolites were separated on an ACQUITY UPLC HSS T3 column (1.7 µm, 50 × 2.1 mm i.d., Waters). The column and autosampler temperatures were maintained at 30°C and 4°C, respectively. The elution condition was set to elute from 0.1% to 50% buffer B in 8 minutes at 0.4 mL/min flow rate, where buffer A comprises of 0.1% (v/v) FA in water and buffer B is 0.1% (v/v) FA in methanol. The injection volume was set to 4 µL.

Raw files from all LC-MS runs were converted to mzXML file format using an open-source software, MSConvert where MS level was set to 1. Converted files (.mzXML) were then processed for retention time adjustment (within lipid spike and between (fatty acid standards) runs) and metabolite matching (HMDB and in-house libraries)⁷¹ using

and in-house built R scripts. The identities of lipid species were verified by MSMS spectra in comparison with commercially available standards (Table 2). Hydroxybutyrate and citrate species were monitored by m/z and retention time (Table 2). The data was processed using Analyst TF 1.7.1, Peakview® 2.2 and MultiQuant 3.0.3 software.

Table 2 Table of parameters of lipid, hydroxybutyrate and citrate species for MS analysis

Lipid Species (Fatty acid chain length)	Parent Mass [M+H]	Fragment Mass
6:0	866.1986	359.1986
8:0	894.2296	387.2296
12:0	950.2935	443.2935
16:0	1006.3583	499.3583
18:0	1034.3889	527.3889
18:1	1032.3721	525.3721
18:2	1030.3599	523.3599
18:3	1028.3407	521.3407
20:0	1062.4198	555.4198
20:4	1054.3443	547.3443
20:5	1052.3397	545.3397
22:0	1090.4471	583.4471
22:6	1078.3569	571.3569

Compound	Parent Mass (m/z)	Retention Time (min)
Unlabeled hydroxybutyrate	103.039	2.33
Labeled ($^{13}\text{C}_4$) hydroxybutyrate	107.0469	2.23
Unlabeled citrate	191.0195	1.84
Labeled ($^{13}\text{C}_2$) citrate	193.0239	1.84

Cell viability assay

Cells were seeded into 96-well Greiner flat-bottomed white plate at a density of 1,000 cells per 50 μ L of media per well. After 24 h, varying concentrations of AZD3965 (Cayman), etomoxir (Selleckchem) and cerulenin (Sigma) were then diluted using media solution to working concentration of 100 μ M, 50 μ M, 25 μ M, 12.5 μ M, 2 μ M, 200 nM and 20 nM. 50 μ L of diluted drug is then added to prior seeded cells, and the final concentrations of drugs are 50 μ M, 25 μ M, 12.5 μ M, 6.25 μ M, 1 μ M, 100 nM and 10 nM respectively. DMSO was used as a negative control. After drug incubation for 72 h, the relative number of viable cells was quantified by measuring adenosine triphosphate (ATP) levels using CellTiter-Glo Luminescent Cell Viability Assay system (Promega, Wisconsin, USA). GI50 values for each cell line are obtained using dose-response curve generated from GraphPad Prism and defined as the concentration of indicated compound required to result in growth inhibitory or lethal response in 50% of the cells.

Cell proliferation assay

Cells were seeded into 96-well plates at a density of 1,000 cells/well for patient-derived NSCLC Lung TIC cell lines and Adh cells. Growth rate is then quantified in 48-h intervals by measuring ATP levels using CellTiter-Glo Luminescent Cell Viability Assay (Promega, Wincosin, USA) system over a duration of 6 to 8 days.

Tumor implantation and collection

5×10^5 single cells, or otherwise stated, were mixed with appropriate growth media, supplements and Matrigel (BD) in a 1:1 ratio and injected subcutaneously into the flanks of 4- to 6-week-old male and female NSG mice (Jackson Laboratories). Mice were sacrificed about 6 weeks later, or when tumor sizes exceeded 1.5 cm in diameter, and tumors were harvested for analysis. High-fat ketogenic diet (Rodent, 5TJQ TestDiet) and standard diet (PicoLab Rodent Diet, #5053) were purchased from LabDiet. Mice were excluded from downstream analysis if weight loss exceeded 15 percent upon change in diet.

All mouse experiments were approved by the Agency for Science, Technology and Research of Singapore–Biological Resource Centre IACUC (protocol number 171286 and 211598). Relevant ethical regulations pertaining to the IACUC protocol were compiled. Written informed consent was obtained from all participating patients

diagnosed with non-small cell adenocarcinoma before surgical resection or biopsy. In brief, tumors were processed in cold PBS with antibiotics, sectioned and chopped with a sterile blade. This is followed by incubation in 1 mg ml⁻¹ collagenase/dispase (Sigma-Aldrich) in DMEM/F12 medium (Thermo Fisher Scientific) at 37 °C with agitation. Suspensions were then washed and reconstituted in PBS and passed through 70- and 40-µm cell strainers to exclude undigested tissues. Cells were then centrifuged and evaluated for cell viability by trypan blue exclusion before downstream assays. Mice were randomized by sex.

Tumor volume measurements

Tumor volume was calculated using the formula $0.5 \times L \times W^2$, where L and W represent tumor length and width, respectively.

Immunoblotting

5 x 10⁵ cells were lysed in Llaemli-SDS buffer and sonicated for 30 seconds with 10 seconds recovery, for a total of 10 cycles. Supernatant of resulting lysates were obtained from pelleting cell debris at 14,800 rotations per minute (rpm) for 30 minutes. Total protein concentration of each sample was determined with Coomassie PlusTM Protein Assay Reagent (ThermoFisher Scientific, Massachusetts, USA) measured at 595nm wavelength. 5µg of proteins from total cell lysates were separated by 4-12% of SDS-PAGE and transferred onto polyvinylidene fluoride (PVDF) membrane, followed by blocking in 5% (v/v) Bovine Serum Albumin (BSA) or milk in Tris-buffered saline with 0.1% Tween-20 (TBST). Proteins were probed with the indicated antibodies and visualized by chemiluminescence (Roche, Basal, Switzerland) using SuperSignal® West Dura Extended Duration Substrate (Thermo Fisher Scientific, Massachusetts, USA).

Primary antibodies used were, anti-GAPDH (Santa Cruz, sc-47724), anti-β-actin (sc-47778), anti-monocarboxylate transporter 1 (MCT1) (sc-365501), anti-monocarboxylate transporter 2 (MCT2) (sc-166925), anti-peroxisome proliferator-activated receptor alpha (PPARα) (sc-398394), anti-peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) (sc-518025), anti-OXCT1 (Abcam, ab70413), anti-ACAT1 (ab110290), anti-ACAT2 (ab74901), anti-CD147 (ab108308),

anti-FASN (sc-55580), anti-CPT1 (sc-393070), anti-EMB (ab127692). The secondary antibodies used were goat anti-mouse and goat anti-rabbit (Santa Cruz).

Lipid droplet staining

Intracellular lipid droplets were visualized with fluorescent BODIPY 493/503 dye (Molecular Probes, Invitrogen, Carlsbad, CA). Cryopreserved tumor sections were thawed or cells in culture were fixed in 4% paraformaldehyde for 15 min at room temperature, rinsed twice with PBS, permeabilized with 0.2% Triton X-100 in PBS for 10 min, and incubated with BODIPY 493/503 dye diluted in PBS (final concentration of 1 μ g/mL) for overnight at 4°C. Tumor sections or cells were washed with PBS and stained with DAPI. Fluorescence images were obtained with an Axio Observer D1 epifluorescence microscope with a built-in AxioCam MR3 camera (Zeiss) using the optimal filters at $\times$ 100 to $\times$ 200 magnifications. The fluorescence intensity of representative images was subject to thresholds and quantified using ImageJ.

Immunofluorescence and immunohistochemistry analyses

Formalin-fixed, paraffin-embedded NSCLC xenograft samples were obtained and cut into 4- μ m sections. NSCLC tissue array with cancer adjacent and normal lung tissue (LC10012b) was purchased from Pantomics. Sections were deparaffinized and antigen retrieval was carried out in citrate buffer in the presence of 0.5% Tween-20. Sections were further permeabilized in 0.2% Triton X-100 and then quenched with TruBlack (Gold Biotechnology). Sections were then incubated with anti-CD147 (ab108308). Alexa Fluor 594 donkey anti-mouse (A21203) and Alexa Fluor 647 goat anti-rabbit (A21245) antibodies from Thermo Fisher were used as secondary antibodies. Fluorescence images were obtained with an Axio Observer D1 epifluorescence microscope with a built-in AxioCam MR3 camera (Zeiss) using the optimal filters at $\times$ 100 to $\times$ 200 magnifications. The fluorescence intensity of representative images was subject to thresholds and quantified using ImageJ.

H&E staining of tumor sections

The tumor sections were fixed overnight in 4% paraformaldehyde, immersed in 70% ethanol, and sent to the Advanced Molecular Pathology Laboratory, IMCB for paraffinization and H&E staining.

sgRNA CRISPR knockout and lentiviral transduction

sgRNAs were cloned into the LentiCRISPRv2GFP lentiviral plasmid (Addgene). Three sgRNAs each were used against CD147 and MCT1. Tumorsphere lines were infected with LentiCRISPRv2GFP lentivirus and sorted for GFP expression 3 d post infection.

Gene	Forward Primer	Reverse primer
Control	CACCGCTGGGTTTGCAATAT GAACG (SEQ ID NO: 1)	AAACCGTTCATATTGCAAACCCAG C (SEQ ID NO: 2)
CD147-1	CACCGGTCGTCAGAACACAT CAACG (SEQ ID NO: 3)	AAACCGTTGATGTGTCTGACGAC C (SEQ ID NO: 4)
CD147-2	CACCGGGATCGCAACCACCT GACCC (SEQ ID NO: 5)	AAACGGGTCAGGTGGTTGCGATC CC (SEQ ID NO: 6)
CD147-3	CACCGGCGAGGAATAGGAA TCATGG (SEQ ID NO: 7)	AAACCCATGATTCCTATTCTCTCGC C (SEQ ID NO: 8)
MCT1-1	CACCGATAGACACAGAATCC AACAT (SEQ ID NO: 9)	AAACATGTTGGATTCTGTGTCTAT C (SEQ ID NO: 10)
MCT1-2	CACCGTATCCATGACACTTC GCTGG (SEQ ID NO: 11)	AAACCCAGCGAAGTGTCATGGAT AC (SEQ ID NO: 12)
MCT1-3	CACCGACAGACGTATAGTTG CTGTA (SEQ ID NO: 13)	AAACTACAGCAACTATACGTCTGT C (SEQ ID NO: 14)

Fluorescence-activated cell sorting

Cells were dissociated with Accutase or 0.05% Trypsin and washed twice with wash buffer (PBS with 2% FBS). Cells were then stained with APC-conjugated anti-CD147 monoclonal antibody (Clone 8D12; Invitrogen, 17-1472-42; 1:250) for 1 hour on ice in the dark and DAPI (4',6-diamidino-2-phenylindole; 1:2000). Stained cells were then washed twice with wash buffer and sieved through a polystyrene tube with cell-strainer caps to ensure suspension of single cells. Cells were resuspended in 200µL of wash buffer in a polypropylene tube before analyzing and sorting for CD147^{high} and CD147^{low} expression (top and bottom 25% of cell population) with LSRII Cell Analyzer (BD). Results were analyzed with FlowJo v10.

RNA extraction, reverse transcription and quantitative polymerase chain reaction (qPCR)

RNA extraction was carried out using TRIzol® Reagent (#15596026), chloroform and RNeasy Mini Kit (50). Reverse transcription was carried out with High-Capacity cDNA Reverse Transcription Kit (ThermoFisher Scientific, Massachusetts, USA) and 2.5 µM anchored oligodT purchased from Integrated DNA Technologies (Iowa, USA). Ribonucleic acid (RNA) templates were incubated with anchored oligodT at 65°C for 5 minutes and placed on ice for 1 minute prior to adding other components of reverse transcription mix to denature RNA secondary structure. qPCR was carried out using Phusion High-Fidelity PCR Master Mix with HF buffer (ThermoFisher Scientific, Massachusetts, USA). Reactions were carried out as per manufacturers' protocol.

Gene	Forward Primer	Reverse primer
BDH1	GGAGCAAGACGCCCACTAT T (SEQ ID NO: 15)	GTCAGTGTTAGGCTGTCC AG (SEQ ID NO: 16)
OXCT1	GTTGGTGGTTTTGGGCTAT GT (SEQ ID NO: 17)	AGACCATGCGTTTATCTG CTT (SEQ ID NO: 18)
ACAT2	GCGGACCATCATAGGTTCC TT (SEQ ID NO: 19)	ACTGGCTTGTCTAACAGGA TTCT (SEQ ID NO: 20)
SCD	TTCCTACCTGCAAGTTCTA CACC (SEQ ID NO: 21)	CCGAGCTTTGTAAGAGCGG T (SEQ ID NO: 22)
SCD5	AGAACATCGTCTGGAGGAA TGTC (SEQ ID NO: 23)	CGACAGCCAGAAATATCCT CA (SEQ ID NO: 24)
FADS2	TGACCGCAAGGTTTACAAC AT (SEQ ID NO: 25)	AGGCATCCGTTGCATCTTC TC (SEQ ID NO: 26)
FASN	ACAGCGGGAATGGGTACT (SEQ ID NO: 27)	GACTGGTACAACGAGCGG AT (SEQ ID NO: 28)
ELOVL6	AACGAGCAAAGTTTGAAC GAGG (SEQ ID NO: 29)	TCGAAGAGCACCGAATATA CTGA (SEQ ID NO: 30)
HMGCS 1	CTCTTGGGATGGACGGTAT GC (SEQ ID NO: 31)	GCTCCAACCTCCACCTGTAG G (SEQ ID NO: 32)

HMGR	TGATTGACCTTTCCAGAGC AAG (SEQ ID NO: 33)	CTAAAATTGCCATTCCACG AGC (SEQ ID NO: 34)
MCT1	TTTCTTTGCGGCTTCCGTTG TTG (SEQ ID NO: 35)	TCAATTTACCCTTCAGCCC CATGG (SEQ ID NO: 36)
CD147	ACATCAACGAGGGGGAGA CG (SEQ ID NO: 37)	GGCTTCAGACAGGCAGGAC A (SEQ ID NO: 38)
GAPDH	GTCAACGGATTTGGTCGTA TT (SEQ ID NO: 39)	GATCTCGCTCCTGGAAGAT GG (SEQ ID NO: 40)

Extracellular acidification rate and oxygen consumption rate measurement

ECAR and OCR measurements were performed by using an XF96 Extracellular Flux analyzer (Seahorse Bioscience) according to the manufacturer's instructions. TICs were plated into XF96 (V3) polystyrene cell culture plates coated with Corning Cell-Tak Cell and Tissue Adhesive at a cell density of 50,000 cells per well on the day of assay. Adherent cells were plated into XF96 (V3) polystyrene cell culture plates at 40,000 cells per well on the day before the assay. Both cell lines were incubated for 24 h in a humidified 37 °C incubator with 5% CO₂ on the day before the assay. During calibration of sensor cartridges, cell plates were incubated in a 37 °C non-CO₂ incubator for 60 min before the start of assay with 175 µl assay medium per well. 25 µl of each compound (oligomycin, FCCP, antimycin A and rotenone) was added to each injection port, and all experiments were performed at 37 °C. ECAR and OCR data points refer to individual rates measured from each well during the measurement cycles and are reported as absolute rates (mpH min⁻¹ or pmol/min). Normalization to cell number with DAPI staining read at 340 nm was then performed to obtain ECAR and OCR values.

Bioinformatics analysis

Gene and protein expression profile of primary LUAD primary tumor and normal tissues from TCGA were analyzed using UALCAN online tool (<http://ualcan.path.uab.edu/analysis.html>). Prognostic significance of 2166 lung cancer and 1161 LUAD patients was analyzed using Kaplan-Meier plotter web server (<http://kmplot.com/analysis/>). Gene expression profiles of treatment-naïve and TKI-resistant cohort are processed using the RSEM expected counts matrices for each cohort,

with DESeq2's TMM-normalisation and limma's batch effect removal, which produces the log counts per million (logCPM) data used in this study.

EXAMPLE 1

Induction of a metabolic switch by tumor-initiating cells to utilize ketone bodies during glucose starvation

Lung TICs promote cancer initiation and contribute towards resistance whereas their non-tumor initiating counterparts drive the rapid proliferation of the tumor mass. The expansion of bulk tumor cells is dependent on the access to, and uptake of, glucose to fuel biosynthetic needs. Consistent with this principle, differentiated non-tumor initiating cells (Adh) were abundant in glycolytic intermediates such as glucose, fructose-6-phosphate and fructose 1,6-bisphosphate when compared to their isogenic TIC counterparts. Because of the lower level of glycolytic intermediates, it was reasoned that glycolytic flux might be lower in TICs. Indeed, measurements of extracellular acidification rate confirmed this observation (**Fig. 1A, left**). Conversely, oxygen consumption rate by mitochondria stress test showed that TICs had a slightly higher mitochondrial respiration rate when compared to their non-TIC counterparts (**Fig. 1A, right**). The results suggested that TICs might be less dependent on glucose but oxidized alternative sources of fuel to meet energy demands. To investigate this possibility, TICs and non-TICs were cultured under progressively lower glucose concentrations in deprivation tests. At 5.5 mM low glucose (LG), corresponding to fasting conditions in human serum, growth of TICs was unaffected and comparable to standard high glucose (HG) cell culture media (**Fig. 1B**). In contrast, the growth of Adh cells was severely stunted when glucose was limited, thus supporting glycolytic flux measurements which pointed to the differential dependency on glucose between functionally different cell types (**Fig. 1B**).

Given that TICs appeared resistant to prolonged fasting levels of glucose, it was sought to determine the minimal glucose concentration threshold at which cellular demands would exceed exogenous availability. Across several concentrations tested, proliferation of TICs was severely crippled at 1.4 mM ultra-low glucose (ULG) for up to 7 days (**Fig. 1C, Fig. 7A**). This corresponds to glucose concentration in poorly perfused regions of

the tumor microenvironment and recapitulates the early events of tumor- or metastasis-initiation during which tumor vasculatures have yet to become established. Hence, during tumor initiation, TICs must adopt alternate carbon sources from the microenvironment when glucose availability is limited. While it was previously demonstrated that high lactate abundance in the tumor microenvironment of human non-small cell lung cancer (NSCLC) could be a preferential source of carbon over glucose, supplementing 10 mM lactate at 1.4 mM of glucose failed to rescue the proliferation of TICs over 7 days (**Fig. 7B**). Thus, at least in the context of tumor initiation, lactate did not appear to be an alternative major metabolite.

To discover metabolic adaptations during glucose limitation, the inventors profiled the transcriptome of TICs cultured in ultra-low glucose for 48 hours and compared gene expression changes to those cultured in high glucose (**Fig. 1D**). 627 genes were differentially expressed; of these, 390 were upregulated and 237 were downregulated. In-depth analysis of metabolism pathways yielded 48 upregulated and 15 downregulated genes. One of the most significantly induced pathways under ULG condition involved the synthesis and degradation of ketone bodies (combined score = 1673, p-value = 1.55×10^{-6}) (**Fig. 1D and Fig. 7C**). Conversely, amino acid and nucleotide sugar metabolism was the top downregulated pathway (combined score = 414, p-value = 5.75×10^{-4}) (**Fig. 7D**). Of the ketone metabolism genes, *BDHI*, *OXCT1* and *ACAT2* were specifically overexpressed in TICs exposed to ULG (**Fig. 1E**). *BDHI* is involved in the interconversion of ketone bodies (acetoacetate and 3-hydroxybutyrate), *OXCT1* catalyzes the rate-limiting step in ketolysis that converts acetoacetate to acetoacetyl-CoA and is normally suppressed in adult tissues, while *ACAT2* catalyzes the reversible conversion of acetoacetyl-CoA to acetyl-CoA with its thiolase activity. As a comparison, the inventors did not observe similar upregulation of ketone metabolism pathways or genes in Adh cells that were subjected to ULG stress (**Fig. 7E-7F**). These observations provided the first indication that TICs, but not their non-TIC counterparts, possessed metabolic adaptations to harness ketones during severe glucose stress.

β -hydroxybutyrate and acetoacetate represent two major ketone bodies produced by the liver through ketogenesis, and are exploited as a source of carbon by several cell types under certain conditions, such as prolonged starvation or as alternative fuel in the brain

³². It may be plausible that the metabolic plasticity seen in normal cell types under stress may extend to cancer cells. To test this, it was examined if ketone supplementation could rescue the growth of TICs exposed to ultra-low glucose (ULG + Acetoacetate). Supplementation of 10 mM acetoacetate rescued the growth of TICs, while doing so had no impact on Adh cells (**Fig. 1F and Fig. 7G**). More crucially, it was sought to evaluate whether the tumorigenic potential of TICs cultured under ultra-low glucose could also be rescued by *ex vivo* ketone supplementation prior to transplantation into immunocompromised NSG mice. Since TICs were able to proliferate for up to 4 days in ultra-low glucose medium, and it was crucial that only viable cells were xenografted. They were cultured for 4 days in either ULG or ULG + Acetoacetate prior to subcutaneous implantation. Consistent with expectations, TICs exposed to ULG were largely inhibited in their tumorigenicity, whereas those supplemented with acetoacetate remained highly tumorigenic (**Fig. 1G**).

As ketones are produced primarily by the liver and their levels are influenced by diet, the inventors investigated whether a systemic increase in circulating ketones would affect the growth of lung TICs *in vivo*. Prior to the transplantation of 5×10^5 TICs, NSG mice had been fed ketogenic diet (composed of 57% fats, 24% protein, 9% fiber and 3% carbohydrates) for 7 days, and subsequently maintained on this diet. Measurements of beta-hydroxybutyrate concentration in circulation after 3 days confirmed these mice exhibited ketosis (**Fig. 1H**). Surprisingly, there was a slight but non-significant increase in tumor growth for mice on ketogenic diet compared to those on standard diet (**Fig. 1H**). This contrasted with tumors generated from 1×10^6 Adh cells, in which tumors exposed to ketogenic diet formed much smaller tumors (**Fig. 1H**). It was reasoned that Adh cells were exquisitely sensitive towards reduced circulating glucose levels resulting from ketogenic diet (**Fig. 1B and Fig. 7G**).

While there was little difference between the size of tumors formed from TICs of ketogenic and standard diet-fed mice, surprisingly, tumors from ketogenic diet-fed mice appeared to bear a more aggressive phenotype, exhibiting invasion capacity into nearby stromal tissue (**Fig. 7I**). This was not seen in tumors formed from Adh cells of ketogenic and standard diet-fed mice (**Fig. 7J**), suggesting that the nature and properties of tumors generated from TICs in mice subjected to different diets might not be the same. To

further explore the functional and phenotypic differences, the inventors performed a tumor repopulation study by re-transplanting 5×10^4 dissociated tumor cells from both ketogenic and standard diet-fed mice into a new batch of mice sustained on standard diet. Very strikingly, re-transplanted tumor cells from mice previously exposed to ketogenic diet retained remarkable tumorigenic potential and gave rise to significantly larger tumors rapidly compared to those previously maintained on standard diet (**Fig. 1I**). The results strongly argued that ketogenic diet might sustain the pool of TICs within primary tumors and explained the marked differences in tumor size following secondary cancer cell transplantation.

Activation of ketolysis drives TCA cycle and lipogenesis

The metabolic switch from glucose to ketone utilization suggested that the latter metabolite might support downstream metabolic needs in the TICs, and in doing so, supplanted glucose as the major source of fuel. As a start, to investigate how ketone utilization remodeled metabolism, the inventors performed global, untargeted metabolomics analyses on TICs cultured under ULG condition or ULG + Acetoacetate (ULG-A) using liquid chromatography-mass spectrometry (LC-MS) (**Fig 2A and Fig. 8A**). Ketolytic intermediates such as acetoacetic acid, acetoacetyl-CoA and 3-hydroxy-3-methylglutarate, were upregulated in TICs cultured under ULG + Acetoacetate; this suggested that TICs could uptake and metabolize ketones (**Fig. 2B**). Notably, supplementation with acetoacetate resulted in a significant upregulation of metabolic intermediates in TCA cycle, ceramide metabolism and fatty acid elongation pathways (**Fig. 2B**). Citrate is a major TCA cycle intermediate that can be transported into cytosol for conversion into acetyl-CoA and malonyl-CoA – both biomolecules are essential for synthesis of fatty acids, thereby pointing to the role of ketones in lipid metabolism, especially towards fatty acid synthesis (**Fig. 2C**).

To unequivocally confirm that acetyl-CoA, arising from ketolysis, was channeled towards the synthesis of long-chained fatty acids, metabolic flux tracing study was performed using β -hydroxybutyrate, which was labelled with carbon-13 (^{13}C - β -hydroxybutyrate). Labelled acetoacetate was not readily available. TICs were exposed to $10\mu\text{M}$ of labelled hydroxybutyrate for 48h under glucose-limiting conditions, and both polar and non-polar fractions were analyzed using LC-MS. ^{13}C - β -hydroxybutyrate

was detected within 30min and peaked at 24h in the cells; this indicated that ketones were readily taken up by TICs (**Fig. 2D**). The fatty acid precursor, ^{13}C -citrate, was also elevated within 30min, and the increase was maintained even at 24h (**Fig. 2E**). In contrast, treating Adh cells with labelled acetoacetate for 48h did not result in any increase in ^{13}C - β -hydroxybutyrate when glucose was limited (**Fig. 8B**). Labelled citrate level was slightly elevated within 30min but decreased after 24h (**Fig. 8C**). Of note, the absolute abundance of labelled citrate that could be detected was low, i.e., 6-fold lower than in TICs. During glucose stress, ketones were channeled towards citrate production and, possibly, fatty acid synthesis in lung TICs.

To connect these cell-based perturbations to the physiological relevance within native tumors, it was sought to investigate if TICs could indeed utilize ketones present either systemically or within the tumor microenvironment for *de novo* lipogenesis. Using excised tumors from mice maintained on either ketogenic or standard diet (**Fig. 1H**), the inventors analyzed the global changes in metabolite profile (**Fig. 8D**). In the aqueous fraction, levels of ketolytic intermediates (3-hydroxy-3-methylglutarate and acetoacetic acid), TCA cycle intermediate (succinic acid) and reduced glutathione were elevated when mice were fed ketogenic diet (**Fig. 2F**). The increased level of reduced glutathione also reflected increased TCA cycle activities. In the non-polar fraction, in support of the notion that circulating ketone bodies could be channeled towards *de novo* lipogenesis, numerous lipid species related to lipogenesis pathway were upregulated (**Fig. 2G**). In contrast, tumors generated from Adh cells did not demonstrate any increase in levels of metabolites associated with the TCA cycle or *de novo* lipogenesis when sustained on ketogenic diet (**Fig. 8E and 8F**). The observations underscored the possibility that Adh cells lacked the capacity to import ketones, or they lacked ketolytic enzymes for efficient ketone metabolism.

To elucidate what the mechanistic adaptations might be, the inventors performed gene expression comparison between TICs and Adh cells. Of the 349 genes which were differentially expressed, 59 were metabolic genes (p -value < 0.05). 39 were upregulated (log fold-change > 1) and 20 were downregulated (log fold-change < -1) in TICs. From pathway analyses (Enrichr; KEGG 2019 Human), biosynthesis of unsaturated fatty acids (combined score = 1523, p -value = 2.09×10^{-7}) and synthesis and degradation of

ketone bodies (combined score = 1174, $-\log(\text{p-value}) = 3.78$) appeared as major pathways upregulated in TICs, relative to Adh cells (**Fig. 9A**). Interestingly, steroid biosynthesis (combined score = 2565, $-\log(\text{p-value}) = 7.33$), which represents another fate of ketone metabolism was also enriched, further highlighting the activation of pathways downstream of ketone metabolism.

The ability of acetoacetate to rescue TIC growth and function indicated that ketolysis and associated downstream pathways were likely functionally intact. First, the expression of key enzymes responsible for ketone catabolism was examined. The rate-limiting enzyme, 3-oxoacid CoA-transferase 1 (OXCT1), mitochondrial acetyl-CoA acetyltransferase (ACAT1), and cytosolic acetyl-CoA acetyltransferase (ACAT2) were abundantly expressed in TICs compared to Adh cells (**Fig. 3A**). This low expression level of OXCT1 in Adh cells likely accounted for their inability to utilize ketones. Strikingly, key lipid metabolism genes responsible for both cellular *de novo* lipogenesis and lipid droplet formation (*FASN*, *ELOVL6*, *HMGCR*, *ACAT1*, *ACAT2*, *SCD*) were also overexpressed in lung TICs, suggesting that lipids might be stored as lipid droplets (**Fig. 3A and 3B**). *De novo* lipogenesis involves synthesis of fatty acids from excess carbohydrates, and fatty acid synthase (*FASN*) is the rate-limiting enzyme, which converts acetyl-CoA and malonyl-CoA into palmitate. Indeed, staining of neutral lipid droplets with BODIPY 493/503 confirmed that they were more abundant in TICs even when glucose was non-limiting, compared to Adh cells (**Fig. 9B**). To profile the composition of lipids, the inventors performed LC-MS lipidomics. Intermediates of *de novo* lipogenesis such as diacylglycerol, triacylglycerol, fatty acids, ceramides, and phospholipids were detected (**Fig. 9C**). During ULG starvation in TICs, lipid droplets were depleted; this is consistent with prior studies, which reported the mobilization of lipid droplets during glucose starvation through lipophagy (**Fig. 9D**). Crucially, however, their abundance could be restored through ketone supplementation, thus pointing to the rapid ability of TICs to activate ketone metabolism for sustaining cellular homeostasis (**Fig. 9D**).

Inhibition of lipogenesis disrupts tumor-initiating cell function

Since lipid metabolism represented a central pathway that was fed through ketolysis, it was reasoned that *de novo* lipogenesis could form a targetable therapeutic axis during

glucose starvation or ketosis. In the presence of acetoacetate supplementation under ULG condition (ULG + Acetoacetate; ULG-A), TICs were 6- and 12-fold more sensitive to cerulenin – a FASN inhibitor – when compared to HG- (GI₅₀: 10.4μM) and ULG-exposure (GI₅₀: 21.3μM), respectively (**Fig. 3C**). Consistent with this, proliferation of TICs was also severely arrested upon exposure to cerulenin under ULG-A condition (**Fig. 3D**). This might be attributed to induced dependency on fatty acid synthesis as a result of ketone availability. To test whether such ‘metabolic addition’ could be exploited for therapeutic intervention, 5 x 10⁵ TICs were transplanted into NSG mice, followed by the administration of either cerulenin (30mg/kg) or vehicle control every 3 days. FASN inhibition clearly arrested tumor growth from TICs after 7 doses of treatment over a period of 18 days (**Fig. 3E**). Next, the inventors evaluated the impact of FASN inhibition when mice xenografted with 5 x 10⁵ TICs were fed ketogenic diet. Very strikingly, tumors were rendered more susceptible towards cerulenin treatment, as evidenced by the inhibition of tumor growth (**Fig. 3F**). The results underscored the tumors’ increased dependence on activated lipogenesis following ketosis in mice. Re-plating of dissociated tumor cells confirmed that TICs from cerulenin-treated mice on ketogenic diet had impaired growth ability during *ex vivo* glucose starvation, as expected (Keto Ceru ULG; **Fig. 9E**). However, when cultured *ex vivo* with standard HG medium, even in the presence of cerulenin, they restored their proliferative capabilities, thus underscoring the metabolic plasticity of these cells (Keto Ceru HG; **Fig. 9E**).

Next, the inventors sought to determine the function of lipogenesis in TICs. A major fate of fatty acid is β-oxidation, whereby its transport into the mitochondria is mediated by carnitine palmitoyltransferase I (CPT1), which converts acyl-coenzyme-A into acyl-carnitines. More recently, β-oxidation has been shown to be an important metabolic axis for supporting specific cell states and metastasis. However, here, no measurable proliferation defect was observed even when a high concentration (> 100μM) of the CPT1-specific inhibitor – etomoxir – was applied to TICs and Adh cells (**Fig. 9F**). As a comparison, the mesenchymal cell line (NAMEC8), which was previously shown to be sensitive to CPT1 inhibition, responded effectively (GI₅₀: 34.3μM). Furthermore, the inventors did not find any significant differences in β-oxidation rate in glucose-starved TICs cultured with or without ketone supplementation (**Fig. 9G**). Thus, fatty acids formed from *de novo* lipogenesis were not utilized for β-oxidation in TICs and suggested

that β -oxidation was not the key fate of fatty acids produced via *de novo* lipogenesis in TICs.

To elucidate the purpose of fatty acid synthesis, it was sought to determine which fatty acid species were essential during glucose stress. Proliferation studies in low glucose media with reduced nutrients (termed basal media, see **Table 3**) revealed that palmitate – the primary product of fatty acid biosynthesis – rescued growth of lung TICs cultured in basal media (**Fig. 9H**). Lipoic acid, an organosulfur derivative of octanoic acid (and hence palmitic acid) with cofactor functions, as well as linoleic acid, a polyunsaturated essential fatty acid for hormone biosynthesis were also able to rescue their growth in basal media when supplemented to lung TICs at 500nM and 150nM, respectively (**Fig. 9H**). Notably, both metabolites are commonly found in nutrient-rich cell culture media. These data suggested that synthesized fatty acids in lung TICs under glucose stress could be important for deriving cofactors important for cell survival and growth.

Monocarboxylate transporter 1 (MCT1) mediates ketone uptake and addiction in the context of ketogenic diet

Having elucidated the fate of ketones in TICs, the inventors investigated the source and consequence of its cellular uptake. In addition to downstream ketolytic and *de novo* lipogenesis enzymes, it was noted that upstream ketone transporter, monocarboxylate transporter 1 (MCT1), was enriched in lung TICs (**Fig. 3A**). In contrast, expression of monocarboxylate transporter 2 (MCT2) and its ancillary protein Embigin (EMB), which also transport ketones, were not elevated in TICs, suggesting that they were unlikely to play a key role (**Fig. 9I**). While paracrine signaling between cells of the tumor compartments has been well-established to promote cancer progression, metabolic crosstalk has also emerged as a major factor. It was speculated that adjacent non-TICs could help modulate ketone availability towards TICs. This led the inventors to investigate the expression levels of master transcriptional activators of ketogenic genes, such as PGC-1 α and PPAR α . PGC-1 α interacts and co-activates PPAR α , among other transcription factors, involved in ketogenesis and fatty acid metabolism. Indeed, both PGC1 α and PPAR α protein expression were higher in differentiated Adh cells as compared to lung TICs (**Fig. 3G**). Furthermore, their target genes such as *ACAT1*, *ACSS1-ACSS3*, *HMGCL* and *HMGCLL1* were specifically upregulated in differentiated

Adh cells under ULG condition, which indicated these cells as a plausible microenvironmental source of ketones (**Fig. 3H and 3I**). In contrast, OXCT2, responsible for ketolytic conversion of acetoacetate to acetoacetyl-CoA became downregulated (**Fig. 3I**).

To evaluate the therapeutic potential of targeting MCT1, the inventors performed dose-response experiment using a clinical trial MCT1 inhibitor, AZD3965, on TICs cultured under ULG condition with or without acetoacetate supplementation. TICs in ULG-A condition (GI_{50} : 1.12 μ M) were more sensitive to AZD3965 when compared to ULG alone (GI_{50} : 8.16 μ M) (**Fig. 3J**). Consistent with this, exposure of lung TICs to AZD3965 in the context of ULG-A arrested their proliferation for up to 7 days *in vitro* (**Fig. 3K**). This reinforced the findings that AZD3965 blocked the import of ketones, which were needed by the TICs under glucose stress. To demonstrate the therapeutic efficacy of AZD3965 in animals, 5×10^5 TICs were transplanted into NSG mice, which were dosed with AZD3965 (50mg/kg/day) or vehicle control, when tumors were established (>5mm). Significant tumor growth inhibition was observed, as expected, on control diet (**Fig. 3L**). Previously, it was noted that ketogenic diet sustained the expansion of the TIC fraction within xenografted tumors, and these tumors showed robust tumor re-initiation potential when secondary repopulation studies were performed (**Fig. 1H and 1I**). With this in mind, it was reasoned that treatment of tumor-bearing mice with AZD3965 in the context of ketogenic diet might further impact primary tumor growth, the composition of TICs within tumors, or their re-population ability. Strikingly, in comparison to standard diet, tumor establishment was dramatically arrested when mice were fed ketogenic diet that enhanced the effects of AZD3965 (**Fig. 3L**). Interestingly, in these tumors, it was also noted that ketogenic diet alone induced an appreciable increase in lipid droplet accumulation (**Fig. 3M and 3N**). Upon AZD3965 administration, there was a 27.8-fold and 13.8-fold reduction in the number of lipid droplets in tumors of ketogenic- and standard diet-fed mice, respectively, thus consistent with the findings on the fate of ketones in lipogenesis. In the context of ketogenic diet, TICs became heavily reliant to ketolysis and were thereby far more sensitive to the effects of AZD3965, which blocked ketone utilization. Ketogenic diet induced a metabolic vulnerability in TICs, and paradoxically enhanced the effects of MCT1 inhibition. This observation mirrored its effects in enhancing the efficacy of PI3K

inhibitors in certain tumors through preventing the activation of PI3K signaling via insulin feedback, thereby providing yet another example for considering the benefits of dietary manipulation in cancer treatment.

Interaction of CD147 with MCT1 is required for membrane localization and ketone uptake

Since MCT1 is an essential conduit for facilitating ketones uptake and their subsequent utilization by TICs, this prompted the inventors to investigate how MCT1 expression and function might be regulated. The chaperone protein, cluster of differentiation 147 (CD147), has been reported to be involved in maturation and localization of MCT1 to the cell surface, but its role in enabling metabolic regulation in cancer has remained largely uninvestigated. The inventors began by knocking out CD147 and MCT1 in TICs using CRISPR-Cas9 (**Fig. 4A and Fig. 10A-C**). Surprisingly, loss of CD147 led to the downregulated expression of MCT1 protein but did not alter its mRNA level (**Fig. 4A and 4B**). Immunofluorescence staining of MCT1 confirmed its substantially reduced protein expression, as well as its retained cytoplasmic localization in CD147 KO TICs, instead of localizing on the plasma membrane when CD147 was present (**Fig. 4C and Fig. 10D**). This indicated that non-membrane bound MCT1 could be regulated post-translationally via proteasomal-dependent degradation. To test this hypothesis, CD147 KO TICs were treated with increasing concentrations of proteasome inhibitor, bortezomib, where the inventors observed a dose-dependent rescue of MCT1 protein expression (**Fig. 4D**). Since TICs were able to mediate a metabolic switch from glucose to ketone dependence quite rapidly, it was examined if the regulation of either CD147 or MCT1 accounted for this metabolic adaptation. Interestingly, under glucose stress, lung TICs upregulated both CD147 and MCT1 expression, thus exemplifying their essentiality in controlling responses towards nutrient regulation (**Fig. 10E**).

To validate the functional relevance of CD147 to MCT1-mediated ketone uptake, the inventors performed metabolic flux tracing studies using ¹³C-labelled β-hydroxybutyrate. Compared to control TICs, CD147 KO significantly reduced the intracellular abundance of ¹³C-labelled β-hydroxybutyrate and ¹³C-labelled citrate, thus confirming that CD147 loss directly impacted MCT1 function and downstream ketone metabolism (**Fig. 4E**). To further demonstrate that the consequence of disrupted ketones

transport was attributed to CD147 loss, proliferation of CD147 KO cells in ULG-A condition was compared to control cells. While control TICs were able to proliferate after day 6, loss of CD147 rendered them impaired (**Fig. 4F**). More importantly, to connect the requirement of CD147 to glucose stress, the inventors showed that re-exposure of high glucose (HG) could effectively rescue the proliferative capacity of CD147 KO cells (**Fig. 10F**). Following transplantation into NSG mice, CD147 KO and MCT1 KO lung TICs were far less tumorigenic – forming much smaller tumors and at a slower rate (**Fig. 4G**). LC-MS analyses further revealed residual tumors formed from CD147 KO TICs had lower abundance of ketolytic and TCA cycle intermediates, as well as fatty acid species (**Fig. 4H and 4I**). Such findings lent weight to the functional importance of ketone uptake and its utilization for fueling the TCA cycle and lipogenesis.

Since genetic ablation of CD147 expression diminished tumorigenic capacity, the inventors next examined if pharmacological inhibition of MCT1 could affect the abundance of CD147⁺ lung TICs and account for their diminished tumorigenic capacity. Using excised tumors from mice on either ketogenic or standard diet, and treated with either vehicle or AZD3965 (**Fig. 3L**), the abundance of CD147⁺ cells was quantified. Consistent with the hypothesis, exposure to ketogenic diet enriched for the abundance of CD147⁺-expressing cells when compared to standard diet, while AZD3965 treatment could abrogate these cells in both dietary contexts (**Fig. 4J**). To further reinforce the metabolic role of CD147, especially in the preclinical context, the inventors generated several patient-derived xenograft lines and profiled its expression (**Fig. 11A**). Of these, A003 and A014 had the most prominent CD147 expression. Strikingly, when mice were subjected to ketogenic diet, both xenograft lines exhibited a more rapid tumor growth (**Fig. 5A and 5B**), which was not associated with changes in weight of ketogenic diet-fed mice (**Fig. 11B and 11C**). Flow cytometry also revealed that the proportion of CD147⁺ cells were more abundant in tumors that were exposed to ketogenic diet, as compared to standard diet (**Fig. 5C**), thereby confirming the notion that ketogenic diet facilitated the maintenance and expansion of the CD147⁺ TIC pool.

The above observations led the inventors to postulate that CD147 could be a functional cell surface biomarker with a distinct metabolic role in TICs. As a start, the inventors

sorted for viable CD147⁺ and CD147⁻ tumor cells from lung xenograft tumors and transplanted 5×10^5 cells of each group into opposite flanks of nude mice. Strikingly, flanks with CD147⁺ cells formed large tumors, while only two out of five flanks bearing CD147⁻ cells formed tumors which were negligible in size (**Fig. 5D**). Next, the inventors asked if the expression of CD147 could have clinical relevance. Comparing between non-small cell lung carcinomas and their cancer-adjacent or normal lung tissues, some cells within tumors expressed high levels of CD147 on the cell surface (**Fig. 5E**). On the other hand, most, if not all, cancer-adjacent tissues did not exhibit CD147 expression (**Fig. 5E**). Statistical analysis across 19 matched pairs of adenocarcinomas and cancer-adjacent or normal samples confirmed that CD147 expression was largely associated with malignant cells (**Fig. 5F**). Likewise, CD147 expression was also more abundant in squamous cell carcinoma (**Fig. 5G**), and largely absent or diffused in normal lung tissue of healthy donors (**Fig. 5F-G, Fig. 11D-E**). This trend was also observed in the TCGA dataset, whereby CD147 gene and protein expression were enriched in primary lung adenocarcinomas (LUAD) (**Fig. 5H and Fig. 11F**). Notably, higher CD147 expression correlated with poorer prognosis (**Fig. 5I and Fig. 11G**).

To further evaluate the association of CD147 in refractory lung cancer, two cohorts of LUAD patients were examined. Gene expression analyses of treatment-naïve and EGFR tyrosine kinase inhibitor (TKI)-resistant tumors indeed confirmed that the latter were more enriched in CD147 expression (**Fig. 5J**). Taken together, the findings revealed a previously unanticipated metabolic switch that is unique to lung TICs, and provided the mechanistic bases for how diet manipulation could alter the course of cancer progression or enhance the efficacy of targeted therapy in a context-dependent manner (**Fig. 6**).

Example 3

Discussion

In overcoming malignant progression, cancer cells encounter changing microenvironments that are created by variations in vasculature, oxygen tension, nutrient availability, as well as through the accumulation of by-products. Subsequent recruitment of microenvironment cell types and heterotypic metabolic exchanges across tumor compartments further diversify the range of metabolic adaptations that cancer

cells may adopt. For instance, in human NSCLC, apart from glucose, lactate can be utilized as an alternate carbon source for TCA cycle. Hypoxic cancer cells can also preferentially metabolize glucose and provide well-oxygenated cancer cells with lactate to drive TCA cycle. However, not all cancer cells within the same tumor respond similarly to glucose limitations. The inventors unexpectedly found that only a subset of lung tumor cells possess innate capacity for a metabolic switch when glucose concentrations were reduced from ~5.5mM (mimicking blood plasma) to ~1mM (recapitulating interstitial fluids). Efforts to translate the targeting of glucose-dependent pathways into effective therapies have yielded limited outcomes. Apart from challenges imposed by metabolic heterogeneity, the majority of current studies also used non-physiological levels of glucose (20-25mM) – a concentration seldom observed in healthy individuals or cancer patients. In this context, the conventional definition of ‘low’ glucose, defined to be ~5.5mM would induce no proliferative defects in bulk lung tumor cells, and insufficient to trigger a metabolic deficit in the tumor-initiating counterparts. Indeed, the use of human plasma-like medium (HPLM) instead of traditional high glucose medium revealed that uric acid, which is 10-fold enriched in human blood, is responsible for inhibiting *de novo* pyrimidine synthesis and subsequent reduction of sensitivity to chemotherapeutic drug 5-fluorouracil in cancer cells. This points to the need to adopt more physiological levels of nutrients to model glucose dependencies to expose the induction of metabolic adaptations across cell types.

Ketones can be utilized as alternative fuel only in a limited spectrum of mammalian tissues during fasting and starvation for channeling into in a myriad of metabolic pathways such as sterol biosynthesis, *de novo* lipogenesis, fatty acid β -oxidation and the TCA cycle. Enhanced ketolysis may be protective against excessive free radical damages and autophagy through reductive reactions and ketone-driven ATP production, providing further selective advantage. Ketogenic diet has long been associated with health benefits, but its pleiotropic effects on cancer progression and therapy response are only emerging. For instance, the risks of implementing ketogenic diet in insulin-deficient diabetic patients are not well-understood. In addition, ketogenic diet alone dampened tumor growth but exacerbated cancer cachexia in IL-6-producing murine cancer models, while administration of dexamethasone in conjunction with ketogenic diet preserved tumor impairment potential and delayed cachexia. Nonetheless,

numerous studies suggested that ketones protect against cancer progression, such as in pancreatic and colon cancer, where ketogenic diet diminished tumor growth and cachexia. Some reports further demonstrate that ketogenic diet may be employed as an adjuvant to conventional chemotherapies. Strikingly, however, glioblastoma, melanoma and hepatocellular carcinoma appear to be ketone-utilizing cancers where ketogenic diet promotes tumor growth.

The findings point to a more nuanced interpretation on the effects of ketogenic diet, in which ketones fuel the expansion of more refractory cancer cells only under glucose stress, ostensibly in poorly vascularized tumor regions where cancer stem-like cells may reside. Paradoxically, this creates a metabolic addiction towards ketones that can be exploited through enhancing the effects of MCT1 inhibitors, at least in preclinical animal models. Other targeted dietary manipulation such as methionine-restriction can sensitize colorectal cancer cells to radiation by effecting changes in one-carbon metabolism. In another study, calorie-restriction, but not ketogenic diet, inhibited PDAC tumor growth because β -hydroxybutyrate could still be incorporated into TCA cycle, and altered lipid availability and fatty acid composition in the tumor cells. Conversely, calorie-restriction was able to reduce levels of almost all fatty acids. The association of diet high in meat products with increased lung cancer risk has been observed across several studies, and recently in EGFR mutant lung cancer. While the complex interplay between protein/fat intake, glucose stress, ketosis and cancer initiation requires further investigation, this study provides a plausible reason to implement dietary modifications early to lower lung cancer risk. Exploitations of diet manipulation in thwarting cancer progression or improving therapy response is a promising intervention modality but information remains patchy. This study provides mechanistic clarity on how metabolic adaptations in relation to intratumoral heterogeneity may be overcome through the implementation of dietary intervention and mechanism-driven clinical trial design.

Table 3: Composition of Basal Media

Components	Molecular Weight	Concentration (mg/L)	mM
Amino Acids			
Glycine	75	30	0.4
L-Arginine hydrochloride	211	84	0.39810428
L-Cystine 2HCl	313	63	0.20127795
L-Glutamine	146	584	4
L-Histidine hydrochloride-H ₂ O	210	42	0.2
L-Isoleucine	181	105	0.8015267
L-Leucine	181	105	0.8015267
L-Lysine hydrochloride	183	146	0.7978142
L-Methionine	149	30	0.20134228
L-Phenylalanine	165	66	0.4
L-Serine	105	42	0.4
L-Threonine	119	95	0.79831934
L-Tryptophan	204	15	0.078431375
L-Tyrosine disodium salt dihydrate	261	104	0.39846742
L-Valine	117	94	0.8034186
Vitamins			
Choline chloride	140	4	0.028571429
D-Calcium pantothenate	477	4	0.008385744
Folic Acid	441	4	0.009070295
Niacinamide	122	4	0.032786893
Pyridoxine hydrochloride	206	4	0.019417476
Riboflavin	376	0.4	0.00106383
Thiamine hydrochloride	337	4	0.011869436
D-Inositol	180	7.2	0.04
Inorganic Salts			
Calcium Chloride (CaCl ₂) (anhyd.)	111	200	1.8018018
Ferric Nitrate (Fe(NO ₃) ₃ ·9H ₂ O)	404	0.1	2.48E-04
Magnesium Sulfate (MgSO ₄) (anhyd.)	120	57.67	0.8139166
Potassium Chloride (KCl)	75	400	5.3333335
Sodium Bicarbonate (NaHCO ₃)	84	3700	44.04762
Sodium Chloride (NaCl)	58	6400	110.344826
Sodium Phosphate monobasic (NaH ₂ PO ₄ ·H ₂ O)	138	125	0.9057971
Other components			
Phenol Red	376.4	15	0.03985122
D-Glucose (Dextrose)	180	990	5.5

Table 4: Supplements for basal media to obtain complete media

Components	Molecular Weight	Concentration (mg/L)	mM
Amino Acids			
Biotin	244	0.0035	1.43E-05
Vitamin B12	1355	0.68	5.02E-04
Hypoxanthine Na	159	2.39	0.015031448
Uroic Acid	280	0.042	1.50E-04
Lipoic Acid	208	0.105	5.10E-04
Putrescine 2HCl	161	0.081	5.03E-04
Thymidine	242	0.365	0.001508265

CLAIMS

1. A method of treating cancer or reducing the risk of recurrence of cancer in a subject, the method comprises contacting a Cluster of Differentiation (CD147) overexpressing cell with an inhibitor of Monocarboxylate Transporter 1 (MCT1) to treat the cancer in the subject.
2. The method of claim 1, wherein the CD147 overexpressing cell is a tumor initiating cell.
3. The method of claim 1 or 2, wherein the CD147 overexpressing cell further overexpresses CD166.
4. The method of any one of claims 1 to 3, wherein the cancer is one that is associated with overexpression of CD147 in one or more tumor initiating cells.
5. The method of any one of claims 1 to 4, wherein the cancer is a lung cancer (such as a refractory lung cancer), a breast cancer or an ovarian cancer.
6. The method of claim 5, wherein the lung cancer is an adenocarcinoma and/or squamous cell carcinoma.
7. The method of any one of claims 1 to 6, wherein the cancer is a ketone-dependent cancer.
8. The method of any one of claims 1 to 7, wherein the subject is one who has been provided a ketogenic diet.
9. The method of any one of claims 1 to 8, wherein the inhibitor of MCT1 is AZD3965, BAY-8002, 7ACC2 or an analogue thereof.
10. The method of any one of claims 1 to 9, wherein the method further comprises contacting the CD147 overexpressing cell with an inhibitor of CD147.

11. The method of claim 10, wherein the inhibitor of CD147 is an antigen-binding molecule.
12. The method of claim 10, wherein the inhibitor of CD147 is Metuzumab, Ac-73 or CD147 antagonist peptide-9 (AP9).
13. The method of any one of claims 1 to 12, wherein the method comprises contacting the CD147 overexpressing cell with an inhibitor of fatty acid synthase (FASN).
14. The method of 13, wherein the inhibitor of FASN is cerulenin, cerulenin derivative C75, orlistat or an analogue thereof
15. The method of any one of claims 1 to 14, wherein inhibiting the tumor initiating cell inhibits tumor growth in the subject.
16. A method of treating cancer or reducing the risk of recurrence of cancer in a subject, wherein the method comprises inhibiting a CD147 overexpressing cell with an inhibitor of MCT1 to treat the cancer or reduce the risk of recurrence of cancer in the subject.
17. The method of claim 16, wherein the method further comprises inhibiting the CD147 overexpressing cell with an inhibitor of CD147.
18. A method of treating cancer or reducing the risk of recurrence of cancer in a subject, wherein the method comprises administering to the subject an inhibitor of MCT1 to treat the cancer or reduce the risk of recurrence of cancer in the subject, wherein the cancer is associated with overexpression of CD147 in one or more tumor initiating cells.
19. The method of claim 18, wherein the method further comprises administering an inhibitor of CD147 to the subject.

20. An inhibitor of MCT1 for use in treating cancer or reducing the risk of recurrence of cancer in the subject, wherein the cancer is associated with overexpression of CD147 in one or more tumor initiating cells.
21. The inhibitor of MCT1 of claim 20, wherein the MCT1 is to be administered with an inhibitor of CD147 to the subject.
22. Use of an inhibitor of MCT1 in the manufacture of a medicament for treating cancer or reducing the risk of recurrence of cancer in the subject, wherein the cancer is associated with overexpression of CD147 in one or more tumor initiating cells.
23. The use of claim 22, wherein the medicament is to be administered with an inhibitor of CD147 to the subject.
24. A method of detecting a cancer that is likely to be susceptible to treatment with an inhibitor of MCT1, the method comprising detecting increased level of expression of CD147 in a sample obtained from the subject, wherein an increased level of expression of CD147 as compared to a reference identifies the subject as one who is suitable for treatment with an inhibitor of ketone metabolism.
25. The method of claim 24, wherein the method comprises detecting increase level of expression of CD147 in one or more cells from a sample obtained from the subject.
26. The method of claim 24 or claim 25, wherein the one or more cells are cancer cells.
27. A method of treating cancer or reducing the risk of recurrence in a subject, the method comprising:
 - a) detecting increased level of expression of CD147 in a sample obtained from the subject, wherein an increased level of expression of CD147 as compared to a reference identifies the subject as one who is suitable for treatment with an inhibitor of MCT1, FASN or CD147, and b) administering an inhibitor of MCT1

to the subject so as to treat the cancer or reduce the risk of recurrence of cancer in the subject.

28. The method of claim 27, wherein the method further comprises administering an inhibitor of CD147 to the subject.

29. A method of treating cancer or reduce the risk of recurrence in a subject, the method comprising:

a) selecting a subject having increased level of expression of CD147 in a sample obtained from the subject, wherein an increased level of expression of CD147 as compared to a reference identifies the subject as one who is suitable for treatment with an inhibitor of MCT1, FASN or CD147, and b) administering an inhibitor of MCT1 to the subject so as to treat the cancer or reduce the risk of recurrence of cancer in the subject.

30. The method of claim 29, wherein the method further comprises administering an inhibitor of CD147 to the subject.

31. A method of stratifying a subject into one who is likely to be suffering from an aggressive or a non-aggressive cancer, the method comprising analyzing the level of CD147 expression in a sample that is obtained from the subject, wherein i) an increased level of expression of CD147 as compared to a reference identifies the subject as one who is likely to be suffering from an aggressive cancer and ii) a lack of increase of expression of CD147 identifies the subject as one who is likely to be suffering from a non-aggressive cancer.

32. A method of predicting a likelihood of a subject who is suffering from an aggressive cancer, wherein the method comprises detecting increased level of expression of CD147 in a sample obtained from the subject, wherein an increased level of expression of CD147 in the sample as compared to a reference indicates that the subject is likely to be suffering from an aggressive cancer.