

OPINION

Fasting and cancer: molecular mechanisms and clinical application

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Abstract | The vulnerability of cancer cells to nutrient deprivation and their dependency on specific metabolites are emerging hallmarks of cancer. Fasting or fasting-mimicking diets (FMDs) lead to wide alterations in growth factors and in metabolite levels, generating environments that can reduce the capability of cancer cells to adapt and survive and thus improving the effects of cancer therapies. In addition, fasting or FMDs increase resistance to chemotherapy in normal but not cancer cells and promote regeneration in normal tissues, which could help prevent detrimental and potentially life-threatening side effects of treatments. While fasting is hardly tolerated by patients, both animal and clinical studies show that cycles of low-calorie FMDs are feasible and overall safe. Several clinical trials evaluating the effect of fasting or FMDs on treatment-emergent adverse events and on efficacy outcomes are ongoing. We propose that the combination of FMDs with chemotherapy, immunotherapy or other treatments represents a potentially promising strategy to increase treatment efficacy, prevent resistance acquisition and reduce side effects.

Dietary and lifestyle-related factors are key determinants of the risk of developing cancer, with certain cancers being more dependent on dietary habits than others^{1–9}. Consistent with this notion, obesity is estimated to account for 14% to 20% of all cancer-related mortality in the United States⁷, leading to guidelines on nutrition and physical activity for reducing the risk of developing cancer⁶. In addition, given the emerging propensity of cancer cells, but not of normal tissues, to disobey anti-growth signals (owing to oncogenic mutations)¹⁰ and their inability to properly adapt to fasting conditions^{11,12}, there is growing interest in the possibility that certain calorie-limited diets could also become an integral part of cancer prevention and, perhaps, of cancer treatment as a means to increase efficacy and tolerability of anticancer agents^{11–13}.

Even though in the past decade we have witnessed unprecedented changes and remarkable advances in cancer treatment^{14,15}, there remains a crucial need for more effective and, possibly, curative

approaches for tumours but also, and just as importantly, for strategies to reduce the side effects of cancer treatments^{15,16}. The issue of treatment-emergent adverse events (TEAEs) is one of the key hurdles in medical oncology^{15,16}. In fact, many patients with cancer experience acute and/or long-term side effects of cancer treatments, which may require hospitalization and aggressive treatments (such as antibiotics, haematopoietic growth factors and blood transfusions) and profoundly affect their quality of life (for example, chemotherapy-induced peripheral neuropathy)¹⁶. Thus, effective toxicity-mitigating strategies are warranted and anticipated to have major medical, societal and economic impact^{15,16}.

Fasting forces healthy cells to enter a slow division and highly protected mode that protects them against toxic insults derived from anticancer drugs while sensitizing different types of cancer cells to these therapeutics^{11,12,17}. This discovery implies that a single dietary intervention could potentially help address different and equally important aspects of cancer

therapy. In this Opinion article, we discuss the biological rationale for using fasting or fasting-mimicking diets (FMDs) to blunt TEAEs but also to prevent and treat cancer. We also illustrate the caveats of this experimental approach^{18,19} and the published and ongoing clinical studies in which fasting or FMDs have been applied to patients with cancer.

Systemic and cellular fasting response

Fasting leads to changes in the activity of many metabolic pathways associated with the switch into a mode able to generate energy and metabolites using carbon sources released primarily from adipose tissue and in part from muscle. The changes in the levels of circulating hormones and metabolites translate into a reduction in cell division and metabolic activity of normal cells and ultimately protect them from chemotherapeutic insults^{11,12}. Cancer cells, by disobeying the anti-growth orders dictated by these starvation conditions, can have the opposite response of normal cells and therefore become sensitized to chemotherapy and other cancer therapies.

Systemic response to fasting. The response to fasting is orchestrated in part by the circulating levels of glucose, insulin, glucagon, growth hormone (GH), IGF1, glucocorticoids and adrenaline. During an initial post-absorptive phase, which typically lasts 6–24 hours, insulin levels start to fall, and glucagon levels rise, promoting the breakdown of liver glycogen stores (which are exhausted after approximately 24 hours) and the consequent release of glucose for energy. Glucagon and low levels of insulin also stimulate the breakdown of triglycerides (which are mostly stored in adipose tissue) into glycerol and free fatty acids. During fasting, most tissues utilize fatty acids for energy, while the brain relies on glucose and on ketone bodies produced by hepatocytes (ketone bodies can be produced from acetyl-CoA generated from fatty acid β -oxidation or from ketogenic amino acids). In the ketogenic phase of fasting, ketone bodies reach concentrations in the millimolar range, typically starting after 2–3 days from the beginning of the fast. Together with fat-derived glycerol and amino acids, ketone bodies fuel

gluconeogenesis, which maintains glucose levels at a concentration of approximately 4 mM (70 mg per dl), which is mostly utilized by the brain. Glucocorticoids and adrenaline also contribute to direct the metabolic adaptations to fasting, helping maintain blood sugar levels and stimulating lipolysis^{20,21}. Notably, although fasting can at least temporarily increase GH levels (to increase gluconeogenesis and lipolysis and to decrease peripheral glucose uptake), fasting reduces IGF1 levels. In addition, under fasting conditions, IGF1 biological activity is restrained in part by an increase in the levels of insulin-like growth factor-binding protein 1 (IGFBP1), which binds to circulating IGF1 and prevents its interaction with the corresponding cell surface receptor²². Finally, fasting decreases the levels of circulating leptin, a hormone predominantly made by adipocytes that inhibits hunger, while increasing the levels of adiponectin, which increases fatty acid breakdown^{23,24}. Thus, in conclusion, the hallmarks of the mammalian systemic response to fasting are low levels of glucose and insulin, high levels of glucagon and ketone bodies, low levels of IGF1 and leptin and high levels of adiponectin.

Cellular response to fasting. The response of healthy cells to fasting is evolutionarily conserved and confers cell protection, and at least in model organisms, has been shown to increase lifespan and healthspan^{12,22,25–31}. The IGF1 signalling cascade is a key signalling pathway involved in mediating the effects of fasting at the cellular level. Under normal nutrition, protein consumption and increased levels of amino acids increase IGF1 levels and stimulate AKT and mTOR activity, thereby boosting protein synthesis. Vice versa, during fasting, IGF1 levels and downstream signalling decrease, reducing AKT-mediated inhibition of mammalian FOXO transcription factors and allowing these transcription factors to transactivate genes, leading to the activation of enzymes such as haem oxygenase 1 (HO1), superoxide dismutase (SOD) and catalase with antioxidant activities and protective effects^{32–34}. High glucose levels stimulate protein kinase A (PKA) signalling, which negatively regulates the master energy sensor AMP-activated protein kinase (AMPK)³⁵, which, in turn, prevents the expression of the stress resistance transcription factor early growth response protein 1 (EGR1) (Msn2 and/or Msn4 in yeast)^{26,36}. Fasting

and the resulting glucose restriction inhibit PKA activity, increase AMPK activity and activate EGR1 and thereby achieve cell-protective effects, including those in the myocardium^{22,25,26}.

Lastly, fasting and FMDs (see below for their composition) also have the ability to promote regenerative effects (BOX 1) by molecular mechanisms, some of which have been implicated in cancer, such as increased autophagy or induction of sirtuin activity^{22,37–49}.

Dietary approaches in cancer

FMDs. The dietary approaches based on fasting that have been investigated more extensively in oncology, both preclinically and clinically, include water fasting (abstinence from all food and drinks except for water) and FMDs^{11,12,17,25,26,50–60} (TABLE 1). Preliminary clinical data indicate that a fast of at least 48 hours may be required to achieve clinically meaningful effects in oncology, such as preventing chemotherapy-induced DNA damage to healthy tissues and helping to maintain patient quality of life during chemotherapy^{52,53,61}. However, most patients refuse or have difficulties completing water fasting, and the potential risks of the extended calorie and micronutrient deficiency associated with it are difficult to justify. FMDs are medically designed dietary regimes very low in calories (that is, typically between 300 and 1,100 kcal per day), sugars and proteins that recreate many of the effects of water-only fasting but with better patient compliance and reduced nutritional risk^{22,61,62}. During an FMD, patients typically receive unrestricted amounts of water, small, standardized portions of vegetable broths, soups, juices, nut bars, and herbal teas, as well as supplements of micronutrients.

In a clinical study of 3 monthly cycles of a 5-day FMD in generally healthy subjects, the diet was well tolerated and reduced trunk and total body fat, blood pressure and IGF1 levels⁶². In previous and ongoing oncological clinical trials, fasting or FMDs have typically been administered every 3–4 weeks, for example, in combination with chemotherapy regimens, and their duration has ranged between 1 and 5 days^{52,53,58,61,63–68}. Importantly, no serious adverse events (level G3 or above, according to Common Terminology Criteria for Adverse Events) were reported in these studies^{52,53,58,61}.

Ketogenic diets. Ketogenic diets (KDs) are dietary regimens that have normal calorie, high-fat and low-carbohydrate content^{69,70}. In a classical KD, the ratio between the

Box 1 | Regenerative effects of fasting and FMDs

Fasting and fasting-mimicking diets (FMDs) can cause substantial regenerative effects in mouse models. Mice fed an FMD starting at 16 months of age for 4 days twice a month show signs of adult neurogenesis, as measured by an increase in the proliferation of immature neurons and by the representation of neural precursors and neural stem cells²². This effect is accompanied by a reduction in circulating and hippocampal IGF1 and in hippocampal protein kinase A (PKA) activity and by a twofold increase in the hippocampal expression of the transcription factor NEUROD1, which is important for neuronal protection and differentiation³⁹. An FMD also led to signs of skeletal muscle rejuvenation in mice — it countered the age-dependent decline in the expression of PAX7, a transcription factor that promotes myogenesis by regulating skeletal muscle satellite cell biogenesis and self-renewal^{122,40}. Periodic fasting also promotes haematopoietic stem cell self-renewal and ameliorates age-dependent myeloid-bias in mice²⁵. IGF1 or PKA deficiency led to similar effects, highlighting a key role for these two signalling pathways in the pro-regenerative effects of fasting in the haematopoietic system. Strikingly, periodic FMD cycles can also promote pancreatic β -cell regeneration, by reducing PKA and mTOR activity and by increasing the expression of developmental markers such as *Nanog*, *Sox17*, *Sox2*, *Ngn3* and *Ins*, followed by *Ngn3*-mediated generation of insulin-producing β -cells⁴¹.

Fasting or FMDs induce autophagy, a naturally occurring, evolutionarily conserved mechanism that disassembles unnecessary or dysfunctional cellular components and allows survival by feeding cell metabolism and repair mechanisms^{22,42,43}. Studies show that autophagy improves healthspan, promotes longevity in mammals and contributes to the lifespan-prolonging effects of calorie-limited diets^{44,45}. In healthy cells, autophagy exerts multiple effects that converge to avoid the risk of malignant transformation, including the preservation of an optimal energetic and redox metabolism, the disposal of potentially harmful and genotoxic molecules, the fight of infections linked to cancer and the preservation of healthy stem cell compartments^{46–48}. A periodic FMD prevented the age-dependent accumulation of p62, a marker of defective autophagy, which suggests that the healthspan-promoting effects of FMDs are carried out at least in part by promotion of autophagic activity²².

Finally, sirtuins, which function as NAD⁺-dependent deacetylases and were ascribed protective and lifespan-extending effects in model organisms, also become more active during fasting^{37,38}. The NAD⁺-producing enzyme nicotinamide phosphoribosyltransferase (NAMPT) and, consequently, intracellular NAD⁺ levels are upregulated during nutrient deprivation as well, further promoting the activity of mitochondrial sirtuins, particularly SIRT3 and SIRT4, and ultimately protecting cells from genotoxic agents, including chemotherapeutics⁴⁹.

Table 1 | Dietary approaches with proposed applications in oncology

Type of diet	Restriction in calories	Composition	Schedule	IGF1 reduction (humans)	Glucose reduction (humans)	Ketone bodies increase (humans)	Location of pro-regenerative effects	Protection from chemotherapy toxicity
Fasting or FMD	>50%	Vegan and low-protein and low-sugar, high-plant-based fat composition, with micronutrient supplementation	Typically 2–5 consecutive days per month	Yes	Yes	Yes	Haematopoietic system, central nervous system, skeletal muscle and pancreatic β -cells (mouse data) ^{22,25,41,153}	Yes (mouse data and DNA damage analyses in patient leukocytes) ^{12,25,26,29,51–53}
Calorie restriction	20–40%	Reduction in all diet constituents except for vitamins and minerals	Chronic	Only in the presence of protein restriction ¹¹⁷	No	No	Intestinal niche stem cells (mouse data) ^{118,119}	Yes (effect lower than that with fasting or FMDs; mouse data) ⁵¹
Ketogenic diet	None (isocaloric)	High-fat, low-carbohydrate composition, with adequate protein content	Chronic	Yes	No	Yes	Peripheral nerves (rat data) ⁸⁷	NA

FMD, fasting-mimicking diet; NA, not available.

weight of fat and the combined weight of carbohydrate and protein is 4:1. Of note, FMDs are also ketogenic because they have high-fat content and have the ability to induce substantial elevations (≥ 0.5 mmol per litre) in the levels of circulating ketone bodies. In humans, a KD can also reduce IGF1 and insulin levels (by more than 20% from baseline values), although these effects are affected by the levels and types of carbohydrates and protein in the diet⁷¹. KDs can reduce blood glucose levels, but they normally remain within the normal range (that is, >4.4 mmol per litre)⁷¹. Notably, KDs may be effective for preventing the increase in glucose and insulin that typically occurs in response to PI3K inhibitors, which was proposed to limit their efficacy⁷². Traditionally, KDs have been used for treating refractory epilepsy, mainly in children⁶⁹. In mouse models, KDs induce anticancer effects, particularly in glioblastoma^{70,72–86}. Clinical studies indicate that KDs probably have no substantial therapeutic activity when used as single agents in patients with cancer and suggest that potential benefits of these diets should be sought in combination with other approaches, such as chemotherapy, radiotherapy, antiangiogenic treatments, PI3K inhibitors and FMDs^{72,73}. KDs were reported to have neuroprotective effects in peripheral nerves and in the hippocampus^{87,88}. However, it remains to be established whether KDs also have pro-regenerative effects similar to fasting or FMDs (BOX 1) and whether KDs also can be used to protect living mammals from the toxicity of chemotherapy. Notably, the

regenerative effects of fasting or FMDs appear to be maximized by the switch from the starvation-response mode, which involves the breakdown of cellular components and the death of many cells, and the re-feeding period, in which cells and tissues undergo reconstruction²². Because KDs do not force entry into a starvation mode, do not promote a major breakdown of intracellular components and tissues and do not include a refeeding period, they are unlikely to cause the type of coordinated regeneration observed during the FMD refeeding.

Calorie restriction. While chronic calorie restriction (CR) and diets deficient in specific amino acids are very different from periodic fasting, they share with fasting and FMDs a more or less selective restriction in nutrients, and they have anticancer effects^{81,89–112}. CR typically involves a chronic 20–30% reduction in energy intake from the standard calorie intake that would allow an individual to maintain a normal weight^{113,114}. It is very effective in reducing cardiovascular risk factors and cancer incidence in model organisms, including primates^{108,109,114}. However, CR can cause side effects, such as changes in physical appearance, increased cold sensitivity, reduced strength, menstrual irregularities, infertility, loss of libido, osteoporosis, slower wound healing, food obsession, irritability and depression. In patients with cancer, there are substantial concerns that it may exacerbate malnutrition and that it will unavoidably cause excessive loss of lean body mass^{18,113–116}. CR reduces fasting blood glucose levels, though they

remain within the normal range¹¹⁴. In humans, chronic CR does not affect IGF1 levels unless a moderate protein restriction is also implemented¹¹⁷. Studies show that by reducing mTORC1 signalling in Paneth cells, CR augments their stem cell function and that it also protects reserve intestinal stem cells from DNA damage^{118,119}, but it is unknown whether pro-regenerative effects in other organs are also elicited by CR. Thus, the available data suggest that fasting and FMDs create a metabolic, regenerative and protective profile that is distinct and probably more potent than that elicited by a KD or CR.

Fasting and FMDs in therapy

Effects on hormone and metabolite levels. Many of the changes in the levels of circulating hormones and metabolites that are typically observed in response to fasting have the capability to exert antitumour effects (that is, reduced levels of glucose, IGF1, insulin and leptin and increased levels of adiponectin)^{23,120,121} and/or to afford protection of healthy tissues from side effects (that is, reduced levels of IGF1 and glucose). Because ketone bodies can inhibit histone deacetylases (HDACs), the fasting-induced increase of ketone bodies may help slow tumour growth and promote differentiation through epigenetic mechanisms¹²². However, the ketone body acetoacetate has been shown to accelerate, instead of reduce, the growth of certain tumours, such as melanomas with mutated BRAF¹²³. Those changes for which there is the strongest evidence for a role in the beneficial effects of fasting and FMDs against cancer are the

reductions in the levels of IGF1 and glucose. At the molecular level, fasting or an FMD reduces intracellular signalling cascades including IGF1R–AKT–mTOR–S6K and cAMP–PKA signalling, increases autophagy, helps normal cells withstand stress and promotes anticancer immunity^{25,29,56,124}.

Differential stress resistance: increasing chemotherapy tolerability.

Some yeast oncogene orthologues, such as Ras and Sch9 (functional orthologue of mammalian S6K), are able to decrease stress resistance in model organisms^{27,28}. In addition, mutations that activate IGF1R, RAS, PI3KCA or AKT, or that inactivate PTEN, are present in the majority of human cancers¹⁰. Together, this led to the hypothesis that starvation would cause opposite effects in cancer versus normal cells in terms of their ability to withstand cell stressors, including chemotherapeutics. In other words, starvation can lead to a differential stress resistance (DSR) between normal and cancer cells. According to the DSR hypothesis, normal cells respond to starvation by downregulating proliferation-associated and ribosome biogenesis and/or assembly genes, which forces cells to enter a self-maintenance mode and shields them from the damage caused by chemotherapy, radiotherapy and other toxic agents. By contrast, in cancer cells, this self-maintenance mode is prevented through

oncogenic changes, which cause constitutive inhibition of stress response pathways¹² (FIG. 1). Consistent with the DSR model, short-term starvation or the deletion of proto-oncogene homologues (that is, Sch9 or both Sch9 and Ras2) increased protection of *Saccharomyces cerevisiae* against oxidative stress or chemotherapy drugs by up to 100-fold as compared with yeast cells expressing the constitutively active oncogene homologue Ras2val19. Similar results were obtained in mammalian cells: exposure to low-glucose media protected primary mouse glia cells against toxicity from hydrogen peroxide or cyclophosphamide (a pro-oxidant chemotherapeutic) but did not protect mouse, rat and human glioma and neuroblastoma cancer cell lines. Consistent with these observations, a 2-day fasting effectively increased the survival of mice treated with high-dose etoposide compared with non-fasted mice and increased the survival of neuroblastoma allograft-bearing mice compared with non-fasted tumour-bearing mice¹².

Subsequent studies found that reduced IGF1 signalling in response to fasting protects primary glia and neurons, but not glioma and neuroblastoma cells, from cyclophosphamide and from pro-oxidative compounds and protects mouse embryonic fibroblasts from doxorubicin²⁹. Liver IGF1-deficient (LID) mice, transgenic animals with a conditional liver *Igf1* gene

deletion that exhibit a 70–80% reduction in circulating IGF1 levels (levels similar to those achieved by a 72-hour fast in mice)^{29,125}, were protected against three out of four chemotherapy drugs tested, including doxorubicin. Histology studies showed signs of doxorubicin-induced cardiac myopathy in only doxorubicin-treated control mice but not in LID mice. In experiments with melanoma-bearing animals treated with doxorubicin, no difference in terms of disease progression between control and LID mice was observed, indicating that cancer cells were not protected from chemotherapy by reduced IGF1 levels. Yet, again, tumour-bearing LID mice exhibited a remarkable survival advantage compared with the control animals owing to their ability to withstand doxorubicin toxicity²⁹. Thus, overall, these results confirmed that IGF1 downregulation is a key mechanism by which fasting increases chemotherapy tolerability.

Both dexamethasone and mTOR inhibitors are widely used in cancer treatment, either because of their efficacy as anti-emetics and anti-allergics (that is, corticosteroids) or for their antitumour properties (that is, corticosteroids and mTOR inhibitors). However, one of their main and frequently dose-limiting side effects is hyperglycaemia. Consistent with the notion that increased glucose–cAMP–PKA signalling reduces resistance to toxicity of chemotherapeutic drugs^{12,26,126}, both dexamethasone and rapamycin increase toxicity of doxorubicin in mouse cardiomyocytes and mice²⁶. Interestingly it was possible to reverse such toxicity by reducing circulating glucose levels through either fasting or insulin injections²⁶. These interventions reduce PKA activity while increasing AMPK activity and thereby activating EGR1, indicating that cAMP–PKA signalling mediates the fasting-induced DSR via EGR1 (REF.²⁶). EGR1 also promotes the expression of cardioprotective peptides, such as the atrial natriuretic peptide (ANP) and the B-type natriuretic peptide (BNP) in heart tissue, which contributes to the resistance to doxorubicin. Furthermore, fasting and/or FMD might protect mice from doxorubicin-induced cardiomyopathy by boosting autophagy, which may promote cellular health by reducing reactive oxygen species (ROS) production through the elimination of dysfunctional mitochondria and by removal of toxic aggregates.

In addition to reducing chemotherapy-induced toxicity in cells and increasing survival of chemotherapy-treated mice, cycles of fasting induce bone

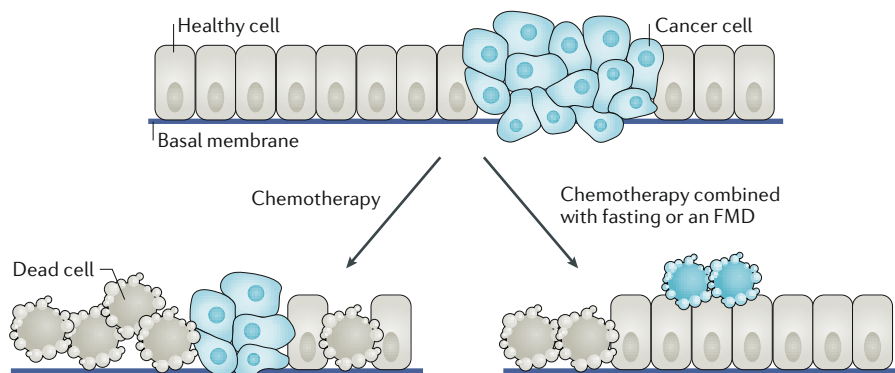


Fig. 1 | Differential stress resistance versus differential stress sensitization. Chemotherapy acts on both cancer cells and normal cells, inducing tumour shrinkage but almost inevitably also causing side effects that can be severe or even life threatening because of the damage to many epithelial and non-epithelial tissues. On the basis of the available preclinical data, fasting or a fasting-mimicking diet (FMD) could prove useful to separate the effects of chemotherapy, and possibly of newer cancer drugs, on normal versus cancer cells. Owing to the presence of oncogenic mutations that constitutively activate growth-promoting signalling cascades, cancer cells fail to properly adapt to starvation conditions. As a result, many types of cancer cells, but not normal cells, experience functional imbalances, becoming sensitized to toxic agents, including chemotherapy (differential stress sensitization). Conversely, fasting or an FMD initiates an evolutionarily conserved molecular response that makes normal cells but not cancer cells more resistant to stressors, including chemotherapy (differential stress resistance). The predicted clinical translation of these differential effects of fasting or FMDs on normal versus cancer cells is a reduction in the side effects of cancer treatments, on the one hand, and improved tumour responses, patient progression-free survival and overall survival, on the other.

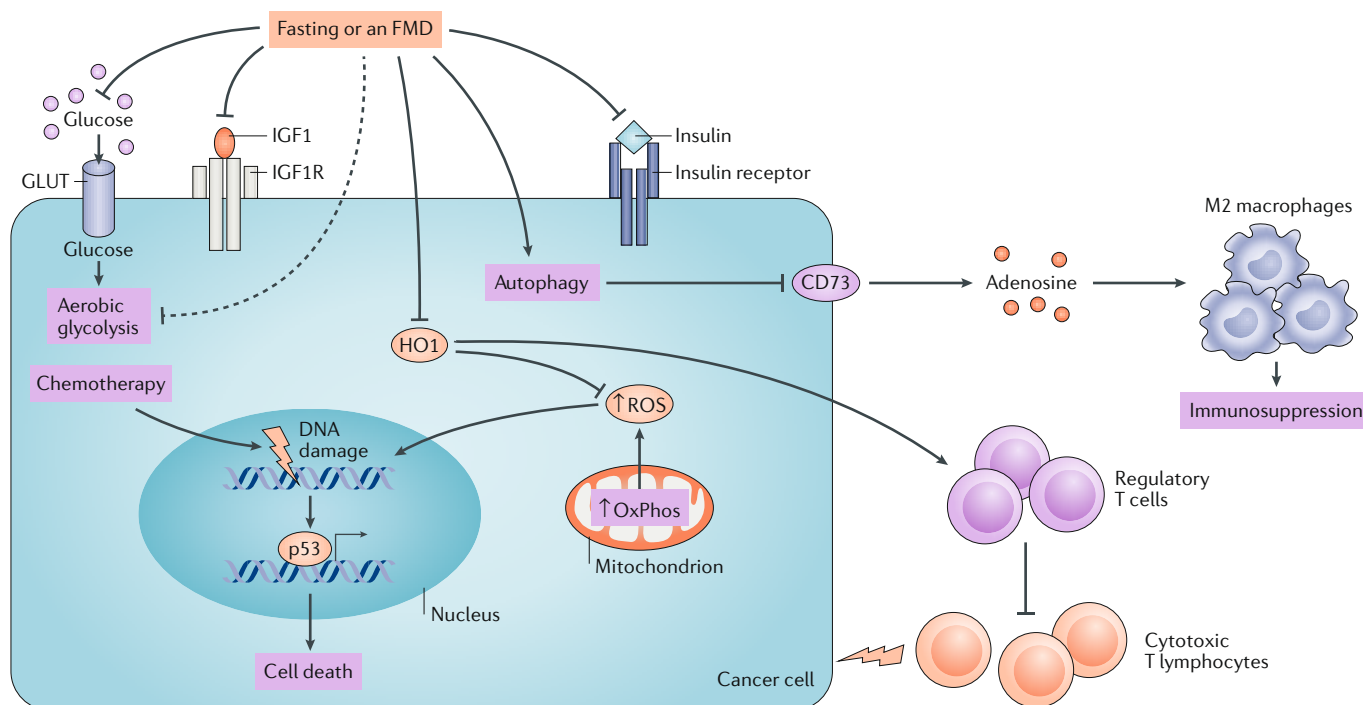


Fig. 2 | Mechanisms of fasting or FMD-dependent killing of cancer cells in solid tumours. Preclinical and initial clinical data indicate that fasting or fasting-mimicking diets (FMDs) reduce the levels of tumour growth-promoting nutrients and factors, including glucose, IGF1 and insulin. Fasting can cause an anti-Warburg effect by reducing glucose uptake via glucose transporters (GLUTs) and aerobic glycolysis and forcing cancer cells to increase oxidative phosphorylation (OxPhos); this increases the production of reactive oxygen species (ROS) in cancer cells and, resultantly, oxidative DNA damage, p53 activation, DNA damage and cell death, particularly in

response to chemotherapy. By activating autophagy, fasting can reduce CD73 levels in some cancer cells, thereby blunting adenosine production in the extracellular environment and preventing the shift of macrophages towards an immunosuppressive M2 phenotype. Finally, fasting or FMDs can downregulate haem oxygenase 1 (HO1) expression in breast cancer cells, which makes them more susceptible to CD8⁺ cytotoxic T cells, possibly by countering the immunosuppressive effect of regulatory T (T_{reg}) cells. Notably, fasting or an FMD can have very different and even opposite effects in different cancer cell types or even within the same cancer cell type.

marrow regeneration and prevent the immunosuppression caused by cyclophosphamide in a PKA-related and IGF1-related manner²⁵. Thus, compelling preclinical results indicate the potential of fasting and FMDs to increase chemotherapy tolerability and to avoid major side effects. Because initial clinical data lend further support to this potential, these preclinical studies build a strong rationale for evaluating FMDs in randomized clinical trials with TEAEs as a primary end point.

Differential stress sensitization: increasing the death of cancer cells. If used alone, most dietary interventions, including fasting and FMDs, have limited effects against cancer progression. According to the differential stress sensitization (DSS) hypothesis, the combination of fasting or FMDs with a second treatment is much more promising^{11,12}. This hypothesis predicts that, while cancer cells are able to adapt to limited oxygen and nutrient concentrations, many types of cancer cells are not able to execute changes that would allow survival in the nutrient-deficient

and toxic environment generated by the combination of fasting and chemotherapy, for example. Early experiments in breast cancer, melanoma and glioma cells found a paradoxical increase in the expression of proliferation-associated genes or of ribosome biogenesis and assembly genes in response to fasting^{11,12}. Such changes were accompanied by unexpected AKT and S6K activation, a propensity to generate ROS and DNA damage and a sensitization to DNA-damaging drugs (via DSS)¹¹. We consider such an inappropriate response of cancer cells to the altered conditions including the reduction in IGF1 and glucose levels caused by fasting or FMDs as a key mechanism underlying the antitumour properties of these dietary interventions and their potential usefulness for separating the effects of anticancer treatments on normal versus malignant cells^{11,12} (FIG. 1). In line with the DSS hypothesis, periodic cycles of fasting or of FMDs are sufficient to slow the growth of many types of tumour cells, ranging from solid tumour cell lines to lymphoid leukaemia cells, in the mouse and, most importantly, to

sensitize cancer cells to chemotherapeutics, radiotherapy and tyrosine kinase inhibitors (TKIs)^{11,17,22,25,50,54–57,59,60,124,127,128}.

By reducing glucose availability and increasing fatty acid β -oxidation, fasting or FMDs can also promote a switch from aerobic glycolysis (Warburg effect) to mitochondrial oxidative phosphorylation in cancer cells, which is necessary for sustaining cancer cell growth in the most nutrient-poor environment⁵⁰ (FIG. 2). This switch leads to increased ROS production¹¹ as a result of increased mitochondrial respiratory activity and may also involve a reduction in cellular redox potential owing to decreased glutathione synthesis from glycolysis and the pentose phosphate pathway⁵⁰. The combined effect of ROS augmentation and reduced antioxidant protection boosts oxidative stress in cancer cells and amplifies the activity of chemotherapeutics. Notably, because a high glycolytic activity demonstrated by high-lactate production is predictive of aggressiveness and metastatic propensity in several types of cancer¹²⁹, the anti-Warburg effects of fasting or FMD have the potential

to be particularly effective against aggressive and metastatic cancers.

Apart from a change in metabolism, fasting or FMDs elicit other changes that can promote DSS in pancreatic cancer cells. Fasting increases the expression levels of equilibrative nucleoside transporter 1 (ENT1), the transporter of gemcitabine across the plasma membrane, leading to improved activity of this drug¹²⁸. In breast cancer cells, fasting causes SUMO2-mediated and/or SUMO3-mediated modification of REV1, a DNA polymerase and a p53-binding protein¹²⁷. This modification reduces the ability of REV1 to inhibit p53, leading to increased p53-mediated transcription of pro-apoptotic genes and, ultimately, to cancer cell demise (FIG. 2). Fasting also increases the ability of commonly administered TKIs to stop cancer cell growth and/or death by strengthening MAPK signalling inhibition and, thereby, blocking E2F transcription factor-dependent gene expression but also by reducing glucose uptake^{17,54}. Finally, fasting can upregulate the leptin receptor and its downstream signalling through the protein PR/SET domain 1 (PRDM1) and thereby inhibit the initiation and reverse the progression of B cell and T cell acute lymphoblastic leukaemia (ALL), but not of acute myeloid leukaemia (AML)⁵⁵. Interestingly, an independent study demonstrated that B cell precursors exhibit a state of chronic restriction in glucose and energy supplies imposed by the transcription factors PAX5 and IKZF1 (REF.¹³⁰). Mutations in the genes encoding these two proteins, which are present in more than 80% of the cases of pre-B cell ALL, were shown to increase glucose uptake and ATP levels. However, reconstituting PAX5 and IKZF1 in pre-B-ALL cells led to an energy crisis and cell demise. Taken together with the previous study, this work indicates that ALL may be sensitive to the nutrient and energy restriction imposed by fasting, possibly representing a good clinical candidate for testing the efficacy of fasting or FMD.

Notably, it is likely that many cancer cell types, including AML²⁹, can acquire resistance by circumventing the metabolic changes imposed by fasting or FMDs, a possibility that is further increased by the metabolic heterogeneity that characterizes many cancers¹²⁹. Thus, a major goal for the near future will be to identify the types of cancer that are most susceptible to these dietary regimens by means of biomarkers. On the other hand, when combined with standard therapies, fasting or FMDs have rarely resulted in the acquisition of resistance

in cancer mouse models, and resistance to fasting combined with chemotherapy is also uncommon in studies *in vitro*, underlining the importance of identifying therapies that, when combined with FMDs, result in potent toxic effects against cancer cells with minimal toxicity to normal cells and tissues^{11,17,50,55–57,59,124}.

Antitumour immunity enhancement by fasting or FMDs. Recent data suggest that fasting or FMDs by themselves, and to a greater extent when combined with chemotherapy, trigger the expansion of lymphoid progenitors and promote tumour immune attack via different mechanisms^{25,56,60,124}. An FMD reduced the expression of HO1, a protein that confers protection against oxidative damage and apoptosis, in cancer cells *in vivo* but upregulated HO1 expression in normal cells^{124,131}. HO1 downregulation in cancer cells mediates FMD-induced chemosensitization by increasing CD8⁺ tumour-infiltrating lymphocyte-dependent cytotoxicity, which may be facilitated by the downregulation of regulatory T cells¹²⁴ (FIG. 2). Another study, which confirmed the ability of fasting or FMDs and CR mimetics to improve anticancer immunosurveillance, implies that the anticancer effects of fasting or FMDs may apply to autophagy-competent, but not autophagy-deficient, cancers⁵⁶. Finally, a recent study of alternate-day fasting for 2 weeks in a mouse colon cancer model showed that, by activating autophagy in cancer cells, fasting downregulates CD73 expression and consequently decreases the production of immunosuppressive adenosine by cancer cells⁶⁰. Ultimately, CD73 downregulation via fasting was shown to prevent macrophage shift to an M2 immunosuppressive phenotype (FIG. 2). On the basis of these studies, it is appealing to speculate that FMDs could be particularly useful instead of or in combination with immune checkpoint inhibitors¹³², cancer vaccines or other drugs that prompt antitumour immunity, including some conventional chemotherapeutics¹³³.

Anticancer diets in mouse models

Overall, the results of preclinical studies of fasting or FMDs in animal cancer models, including models for metastatic cancer (TABLE 2), show that periodic fasting or FMDs achieve pleiotropic anticancer effects and potentiate the activity of chemotherapeutics and TKIs while exerting protective and regenerative effects in multiple organs^{22,25}. Achieving the same

effects without fasting and/or FMDs would require first the identification and then the use of multiple effective, expensive and frequently toxic drugs and would probably be without the advantage of inducing healthy cell protection. It is noteworthy that in at least two studies fasting combined with chemotherapy proved to be the only intervention capable of achieving either complete tumour regressions or long-term survival in a consistent fraction of the treated animals^{11,59}.

Chronic KDs also show a tumour growth-delaying effect when used as a monotherapy, particularly in brain cancer mouse models^{77,78,80–82,84,134}. Gliomas in mice maintained on a chronic KD have reduced expression of the hypoxia marker carbonic anhydrase 9 and of hypoxia-inducible factor 1 α , decreased nuclear factor- κ B activation and reduced vascular marker expression (that is, vascular endothelial growth factor receptor 2, matrix metalloproteinase 2 and vimentin)⁸⁶. In an intracranial mouse model of glioma, mice fed a KD exhibited increased tumour-reactive innate and adaptive immune responses that were primarily mediated by CD8⁺ T cells⁷⁹. KDs were shown to improve the activity of carboplatin, cyclophosphamide and radiotherapy in glioma, lung cancer and neuroblastoma mouse models^{73–75,135}. In addition, a recent study shows that a KD could be very useful in combination with PI3K inhibitors⁷². By blocking insulin signalling, these agents promote glycogen breakdown in the liver and prevent glucose uptake in the skeletal muscle, which leads to transient hyperglycaemia and to a compensatory insulin release from the pancreas (a phenomenon known as 'insulin feedback'). In turn, this raise in insulin levels, which can be protracted, particularly in patients with insulin resistance, reactivates PI3K–mTOR signalling in tumours, thus strongly limiting the benefit of PI3K inhibitors. A KD was shown to be very effective at preventing insulin feedback in response to these drugs and to strongly improve their anticancer activity in the mouse. Finally, according to a study in a murine tumour-induced cachexia model (MAC16 tumours), KDs could help prevent the loss of fat and non-fat body mass in patients with cancer⁸⁵.

CR reduced tumorigenesis in genetic mouse cancer models, mouse models with spontaneous tumorigenesis and carcinogen-induced cancer mouse models, as well as in monkeys^{91,92,97,98,101,102,104–106,108,109,136–138}. By contrast, a study found that CR from middle age actually increases the incidence of plasma cell neoplasms in

Table 2 | Fasting or FMDs in cancer mouse models

Cancer model	Mouse strains	Dietary regimen	Main findings	Refs
Metastatic neuroblastoma model (intravenous cancer cell injection): NXS2 (mouse neuroblastoma allograft)	A/J, CD-1 and athymic nude mice	48 h fasting (water only) given once prior to high-dose etoposide injection versus ad libitum diet	Fasting cycles reduced toxicity of high-dose etoposide in mice but did not reduce etoposide activity profile against neuroblastoma allografts	12
Subcutaneous tumour models: 4T1 (mouse breast cancer allograft), B16 (mouse melanoma allograft), GL26 (mouse glioma allograft), ACN (human neuroblastoma xenograft), MDA-MB-231 (human breast cancer xenograft) and OVCAR3 (human ovarian cancer xenograft). Metastatic cancer models (intravenous cancer cell injection): 4T1 (allograft), B16 (allograft), NXS2 (mouse neuroblastoma allograft) and Neuro-2a (mouse neuroblastoma allograft)	BALB/c, C57Bl/6 and athymic nude mice	48 h fasting (water only) given once a week between 1 and 4 times, 24 h prior to and 24 h after chemotherapy injection versus ad libitum diet	Fasting cycles combined with doxorubicin or cyclophosphamide were superior to each treatment alone in retarding the growth of subcutaneously growing tumours and extending survival in metastatic models of breast cancer, melanoma and neuroblastoma	11
Subcutaneous tumour models: ZL55 (human mesothelioma xenograft) and A549 (human lung cancer xenograft)	Nude mice	48 h fasting (water only) given once a week for 3 times, 32 h prior to and 16 h after cisplatin injection versus ad libitum diet	Fasting sensitized human mesothelioma and lung cancer xenografts to cisplatin. Complete remissions were observed in only the combination treatments (in 40–60% of the mice)	59
Subcutaneous tumour models: H2133 (human lung cancer xenograft) and HCT116 (human colorectal cancer xenograft)	Athymic nude mice	48 h fasting (water only) given once a week for 3 times during daily treatment with crizotinib or regorafenib versus ad libitum diet	Fasting improved the clinical activity of crizotinib and of regorafenib and boosted their ability to block MAPK signalling	17
Subcutaneous tumour model: CT26 (mouse colon cancer allograft)	BALB/c mice	48 h fasting (water only) given once a week for 2 times, 24 h prior to and 24 h after oxaliplatin injection versus ad libitum diet	Fasting potentiated the anticancer effects of oxaliplatin, exerted anti-Warburg effects and promoted oxidative stress and apoptosis in cancer cells	50
Subcutaneous tumour models: 4T1 (mouse breast cancer allograft), B16 (mouse melanoma allograft) and MCF7 (human breast cancer xenograft)	BALB/c, C57Bl/6 and athymic nude mice	48–60 h fasting (water only) or a 96 h FMD given once a week for 2 to 4 times versus ad libitum diet. Animals were injected with chemotherapy at the end of each fasting and/or FMD cycle	An FMD was as effective as fasting at reducing tumour progression when combined with doxorubicin or cyclophosphamide. The FMD downregulated HO1 expression in cancer cells, expanded lymphoid progenitors in the bone marrow and boosted anticancer immunity	124
Subcutaneous tumour model: MCA205 (mouse fibrosarcoma allograft)	C57Bl/6 and athymic nude mice	48 h fasting (water only) given once versus ad libitum diet. Animals were injected with mitoxantrone or oxaliplatin at the end of fasting	Fasting and calorie restriction mimetics improved the efficacy of chemotherapy in an immune system-dependent and autophagy-dependent fashion. Autophagy was shown to allow for optimal release of ATP from dying cancer cells, leading to the depletion of intratumoural regulatory T cells and thereby improving the anticancer immune response	56
B-ALL, T-ALL and AML models: Lin [−] bone marrow cells were infected with retroviruses expressing MYC-IRES-GFP (B-ALL), NOTCH1-IRES-GFP (T-ALL) or MLL-AF9-IRES-YFP (AML) and subsequently transplanted into irradiated mice	C57Bl/6 mice	1 day of fasting followed by 1 day of feeding, for a total of 6 cycles starting from day 2 after transplantation versus ad libitum diet	Fasting inhibited B-ALL and T-ALL development by upregulation of the leptin receptor and its downstream signalling. AML growth was not affected	55
Subcutaneous tumour model: CT26 (mouse colon cancer allograft)	BALB/c mice	24 h fasting on alternate days for 2 weeks	Fasting inhibited colon cancer growth and decreased the production of extracellular adenosine by cancer cells by suppressing CD73 expression	60
Subcutaneous tumour model: BxPC-3 (human pancreatic cancer xenograft)	Nu/Nu nude mice	24 h fasting (water only) before the administration of gemcitabine	Fasting before gemcitabine injection delayed pancreatic cancer progression and increased tumour ENT1 levels	128

Table 2 (cont.) | **Fasting or FMDs in cancer mouse models**

Cancer model	Mouse strains	Dietary regimen	Main findings	Refs
p53 ^{+/-} mice; these mice are prone to spontaneous neoplasms (most commonly sarcoma and lymphoma)	C57Bl/6 mice	24 h fasting (water only) once a week	Fasting delayed the onset of tumours in adult mice and lowered leptin and IGF1 compared with mice fed ad libitum	89
Age-associated lymphoma	OF-1 mice	Alternate-day fasting initiated at 8 months of age through a 4-month period	Fasting reduced the incidence of lymphoma (0% versus 33% for controls), decreased the mitochondrial generation of ROS and increased spleen mitochondrial SOD activity	145

B-ALL, B cell acute lymphoblastic leukaemia; ENT1, equilibrative nucleoside transporter 1; FMD, fasting-mimicking diet; GFP, green fluorescent protein; HO1, haem oxygenase 1; ROS, reactive oxygen species; SOD, superoxide dismutase; T-ALL, T cell acute lymphoblastic leukaemia; YFP, yellow fluorescent protein.

C57Bl/6 mice¹³⁹. However, in the same study, CR also extended maximum lifespan by approximately 15%, and the observed increase in cancer incidence was attributed to the increased longevity of mice undergoing CR, the age at which tumour-bearing mice undergoing CR died and the percentage of tumour-bearing mice undergoing CR that died. Thus, the authors concluded that CR probably retards promotion and/or progression of existing lymphoid cancers. A meta-analysis comparing chronic CR with intermittent CR in terms of their ability to prevent cancer in rodents concluded that intermittent CR is more effective in genetically engineered mouse models, but it is less effective in chemically induced rat models⁹⁰. CR was shown to slow tumour growth and/or to extend mouse survival in various cancer mouse models, including ovarian and pancreatic cancer^{140,94} and neuroblastoma⁸¹. Importantly, CR improved the activity of anticancer treatment in several cancer models, including the activity of an anti-IGF1R antibody (ganitumab) against prostate cancer¹⁴¹, cyclophosphamide against neuroblastoma cells¹³⁵ and autophagy inhibition in xenografts of HRAS-G12V-transformed immortal baby mouse kidney epithelial cells¹⁰⁰. However, CR or a KD in combination with anticancer therapies seems to be less effective than fasting. A mouse study found that, in contrast to fasting alone, CR alone was not able to reduce the growth of subcutaneously growing GL26 mouse gliomas and that, again, in contrast to short-term fasting, CR did not increase cisplatin activity against subcutaneous 4T1 breast tumours⁵¹. In the same study, fasting also proved substantially more effective than CR and a KD at increasing the tolerability of doxorubicin⁵¹. Although fasting or an FMD, CR and a KD likely act on and modulate overlapping signalling pathways, fasting or an FMD probably affects such mechanisms in a more drastic fashion during an intense acute phase of a maximum duration of a few days. The phase of refeeding could

then favour the recovery of homeostasis of the whole organism but also activate and invigorate mechanisms that can promote the recognition and removal of the tumour and regenerate the healthy cells. CR and a KD are chronic interventions that are able to only moderately repress nutrient-sensing pathway, possibly without reaching certain thresholds necessary to improve the effects of anticancer drugs, while imposing a major burden and often a progressive weight loss. CR and a KD as chronic dietary regimens in patients with cancer are difficult to implement and likely bear health risks. CR would likely lead to severe loss of lean body mass and the reduction of steroid hormones and possibly immune function¹⁴². Chronic KDs are also associated with similar although less severe side effects¹⁴³. Thus, periodic fasting and FMD cycles lasting less than 5 days applied together with standard therapies have a high potential to improve cancer treatment while reducing its side effects. Notably, it will be important to study the effect of the combination of periodic FMDs, chronic KDs and standard therapies, particularly for the treatment of aggressive cancers such as glioma.

Fasting and FMDs in cancer prevention

Epidemiological studies and studies in animals, including monkeys^{108,109,144}, and humans lend support to the notion that chronic CR and periodic fasting and/or an FMD could have cancer-preventive effects in humans. Nevertheless, CR can hardly be implemented in the general population owing to issues of compliance and to possible side effects¹¹⁵. Thus, while evidence-based recommendations of foods to prefer (or to avoid) as well as lifestyle recommendations to reduce cancer risk are becoming established^{6,8,9,15}, the goal now is to identify and, possibly, standardize well tolerated, periodic dietary regimens with low or no side effects and evaluate their cancer-preventive efficacy in clinical studies. As discussed earlier, FMD cycles cause downregulation of IGF1 and glucose

and upregulation of IGFBP1 and ketone bodies, which are changes similar to those caused by fasting itself and are biomarkers of the fasting response²². When C57Bl/6 mice (which spontaneously develop tumours, primarily lymphomas, as they age) were fed such an FMD for 4 days twice a month starting at middle age and an ad libitum diet in the period between FMD cycles, the incidence of neoplasms was reduced from approximately 70% in mice on the control diet to approximately 40% in mice in the FMD group (an overall 43% reduction)²². In addition, the FMD postponed by over 3 months the occurrence of neoplasm-related deaths, and the number of animals with multiple abnormal lesions was more than threefold higher in the control group than in the FMD mice, indicating that many tumours in the FMD mice were less aggressive or benign. A previous study of alternate-day fasting, which was performed in middle-aged mice for a total of 4 months, also found that fasting reduced the incidence of lymphoma, bringing it from 33% (for control mice) to 0% (in fasted animals)¹⁴⁵, although because of the short duration of the study it is unknown whether this fasting regimen prevented or simply delayed the tumour onset. Furthermore, alternate-day fasting imposes 15 days per month of complete water-only fasting, whereas in the FMD experiment described above mice were placed on a diet that provided a limited amount of food for only 8 days per month.

In humans, 3 cycles of a 5-day FMD once a month were shown to reduce abdominal obesity and markers of inflammation as well as IGF1 and glucose levels in subjects with elevated levels of these markers⁶², indicating that periodic use of an FMD could potentially have preventive effects for obesity-related or inflammation-related, but also other, cancers in humans, as it has been shown for mice²². Therefore, the promising results of preclinical studies combined with the clinical data on the effect of an FMD on risk factors for ageing-associated diseases, including cancer⁶², lend support to future

randomized studies of FMDs as a possibly effective tool to prevent cancer, as well as other ageing-associated chronic conditions, in humans.

Clinical applicability in oncology

Four feasibility studies of fasting and FMDs in patients undergoing chemotherapy have been published as of today^{52,53,58,61}. In a case series of 10 patients diagnosed with various types of cancer, including breast, prostate, ovarian, uterus, lung and oesophageal cancer, who voluntarily fasted for up to 140 hours before and/or up to 56 hours following chemotherapy, no major side effects caused by fasting itself other than hunger and lightheadedness were reported⁵⁸. Those patients (six) who underwent chemotherapy with and without fasting reported a significant reduction in fatigue, weakness and gastrointestinal adverse events while fasting. In addition, in those patients in which cancer progression could be assessed, fasting did not prevent chemotherapy-induced reductions in tumour volume or in tumour markers.

In another study, 13 women with HER2 (also known as ERBB2) negative, stage II/III

breast cancer receiving neo-adjuvant taxotere, adriamycin and cyclophosphamide (TAC) chemotherapy were randomized to fast (water only) 24 hours before and after beginning chemotherapy or to nutrition according to standard guidelines⁵². Short-term fasting was well tolerated and reduced the drop in mean erythrocyte and thrombocyte counts 7 days after chemotherapy. Interestingly, in this study, the levels of γ -H2AX (a marker of DNA damage) were increased 30 minutes after chemotherapy in leukocytes from non-fasted patients but not in patients who had fasted.

In a dose escalation of fasting in patients undergoing platinum-based chemotherapy, 20 patients (who were primarily treated for either urothelial, ovarian or breast cancer) were randomized to fast for 24, 48 or 72 hours (divided as 48 hours before chemotherapy and 24 hours after chemotherapy)⁵³. Feasibility criteria (defined as three or more out of six subjects in each cohort consuming ≤ 200 kcal per day during the fast period without excess toxicity) were met. Fasting-related toxicities were always grade 2 or below, the most common being fatigue, headache and dizziness. As in

the previous study, reduced DNA damage (as detected by comet assay) in leukocytes from subjects who fasted for at least 48 hours (as compared with subjects who fasted for only 24 hours) could also be detected in this small trial. In addition, a nonsignificant trend towards less grade 3 or grade 4 neutropenia in patients who fasted for 48 and 72 hours versus those who fasted for only 24 hours was also documented.

Very recently, a randomized crossover clinical trial was conducted assessing the effects of an FMD on quality of life and side effects of chemotherapy in a total of 34 patients with breast or ovarian cancer⁶¹. The FMD consisted of a daily caloric intake of <400 kcal, primarily by juices and broths, starting 36–48 hours before the beginning of chemotherapy and lasting until 24 hours after the end of chemotherapy. In this study, the FMD prevented the chemotherapy-induced reduction in quality of life and it also reduced fatigue. Again, no serious adverse events of the FMD were reported.

Several other clinical trials of FMDs in combination with chemotherapy or with other types of active treatments are currently ongoing at US and European hospitals,

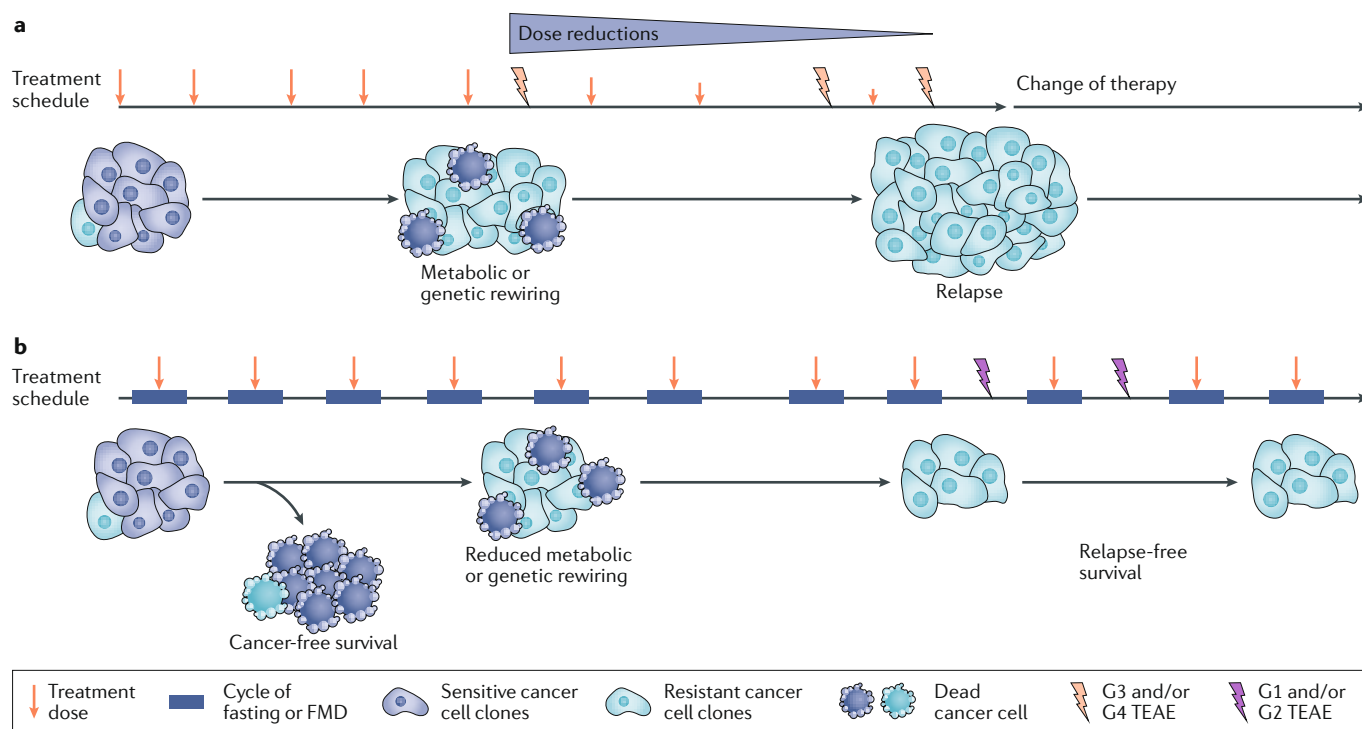


Fig. 3 | Working hypothesis for the effects of the combination of fasting and/or FMDs with standard therapy in oncology. a | The benefit of cancer treatments is limited by the development of resistance to the agents that are employed but also by treatment-emergent adverse events (TEAEs), which can be severe or even life threatening and may require hospitalization (G3 and/or G4 TEAEs according to Common Terminology Criteria for Adverse Events). Disease progression under treatment and G3 and/or G4 TEAEs are the main causes of treatment discontinuations and of the switch to other lines of treatment or to palliative care. **b** | Fasting-mimicking diets (FMDs) combined with standard treatments are predicted to increase the ability of the latter to be curative or, at least, to delay the emergence of resistant cancer cell clones. In addition, cycles of fasting or FMDs are anticipated to reduce treatment toxicity, possibly switching G3 and/or G4 TEAEs to less severe G1 and/or G2 TEAEs, and to help patients maintain their quality of life throughout therapy.

primarily in patients who are diagnosed with breast or prostate cancer^{63,65–68}. These are either one-arm clinical studies to assess FMD safety and feasibility or randomized clinical studies focusing either on the effect of the FMD on the toxicity of chemotherapy or on the quality of life of patients during chemotherapy itself. Altogether, these studies have now enrolled over 300 patients, and their first results are expected to become available in 2019.

Challenges in the clinic. The study of periodic fasting or of FMDs in oncology is not devoid of concerns, particularly in relation to the possibility that this type of dietary regimen could precipitate malnutrition, sarcopenia and cachexia in predisposed or frail patients (for example, patients who develop anorexia as a consequence of chemotherapy)^{18,19}. However, no instances of severe (above grade 3) weight loss or of malnutrition were reported in the clinical studies of fasting in combination with chemotherapy published as of now, and those patients who did experience a weight loss during fasting typically recovered their weight before the subsequent cycle without detectable harm. Nevertheless, we recommend that periodic anorexia and nutritional status assessments using gold-standard approaches^{18,19,146–150} should be an integral part of these studies and that any ensuing nutritional impairment in patients undergoing fasting and/or FMDs is rapidly corrected.

Conclusions

Periodic fasting or FMDs consistently show powerful anticancer effects in mouse cancer models including the ability to potentiate chemoradiotherapy and TKIs and to trigger anticancer immunity. FMD cycles are more feasible than chronic dietary regimens because they allow patients to consume food regularly during the FMD, maintain a normal diet between cycles and do not result in severe weight loss and possibly detrimental effects on the immune and endocrine systems. Notably, as standalone therapies, periodic fasting or FMD cycles would probably show limited efficacy against established tumours. In fact, in mice, fasting or FMDs affect the progression of a number of cancers similarly to chemotherapy, but alone, they rarely match the effect obtained in combination with cancer drugs which can result in cancer-free survival^{11,59}. Thus, we propose that it is the combination of periodic FMD cycles with standard treatments that holds the highest potential to promote cancer-free survival in patients,

as suggested by the mouse models^{11,59} (FIG. 3). This combination may be particularly potent for several reasons: first, cancer drugs and other therapies can be effective, but a portion of patients do not respond because cancer cells adopt alternative metabolic strategies leading to survival. These alternative metabolic modes are much more difficult to sustain under fasting or FMD conditions because of the deficiencies or changes in glucose, certain amino acids, hormones and growth factors, as well as in other unknown pathways leading to cell death. Second, fasting or FMDs can prevent or reduce resistance acquisition. Third, fasting or FMDs protect normal cells and organs from the side effects caused by a wide variety of cancer drugs. On the basis of preclinical and clinical evidence of feasibility, safety and efficacy (at reducing IGF1, visceral fat and cardiovascular risk factors), FMDs also appear as a viable dietary approach to be studied in cancer prevention.

An important future challenge will be to identify those tumours that are the best candidates to benefit from fasting or FMDs. Even in cancer types that are apparently less responsive to fasting or FMDs, it may still be possible to identify the mechanisms of resistance and to intervene with drugs able to revert that resistance. Conversely, more caution should be adopted with other types of diets, especially if high in calories, as they could lead to exacerbated and not inhibited growth of certain cancers. For example, the KD increases growth of a melanoma model with mutated BRAF in mice¹²³, and it was also reported to accelerate disease progression in a mouse AML model⁷². Furthermore, it is essential to apply FMDs with an understanding of the mechanisms of action, since their potency if applied incorrectly could generate negative effects. For example, when rats were fasted and treated with a potent carcinogen before refeeding, this resulted in the growth of aberrant foci in liver, colon and rectum when compared with non-fasted rats^{151,152}. Although the mechanisms involved in this effect are not understood, and these foci may have not resulted in tumours, these studies suggest that a minimum period of 24–48 hours between the chemotherapy treatment and the return to the normal diet is important to avoid combining the regrowth signals present during the refeeding after fasting with high levels of toxic drugs such as chemotherapy.

The clinical studies of fasting or FMD in patients undergoing chemotherapy support its feasibility and overall safety^{52,53,58,61}. In a small-size randomized trial that enrolled 34

patients, an FMD helped patients maintain their quality of life during chemotherapy and reduced fatigue⁶¹. In addition, preliminary data suggest the potential of fasting or FMDs to reduce chemotherapy-induced DNA damage in healthy cells in patients^{52,53}. Ongoing clinical studies of FMDs in patients with cancer^{63,65–68} will provide more solid answers as to whether prescribing periodic FMDs in combination with conventional anticancer agents helps improve tolerability and activity of the latter. It is important to consider that FMDs will not be effective in reducing the side effects of cancer treatments in all patients and neither will they work to improve the efficacy of all therapies, but they have great potential to do so at least for a portion and possibly for a major portion of patients and drugs. Frail or malnourished patients or patients at risk of malnutrition should not be enrolled in clinical studies of fasting or FMDs, and patient nutritional status and anorexia should be carefully monitored throughout clinical trials. An appropriate intake of proteins, essential fatty acids, vitamins and minerals combined, where possible, with light and/or moderate physical activity aimed at increasing muscle mass should be applied between fasting or FMD cycles in order for the patients to maintain a healthy lean body mass^{18,19}. This multimodal dietary approach will maximize the benefits of fasting or FMDs while at the same time protecting patients from malnutrition.

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Author contributions

V.D.L. substantially contributed to discussion of content, wrote the manuscript and reviewed and/or edited it before submission. A.N. researched data for the manuscript, substantially contributed to discussion of content and wrote the manuscript. I.C. and S.C. researched data for the manuscript and reviewed and/or edited the manuscript before submission.

Competing interests

A.N. and I.C. are inventors on three patents of methods for treating cancer by fasting-mimicking diets that are currently under negotiation with L-Nutra Inc. V.D.L. is the founder of L-Nutra Inc.

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