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Current Opinion in Biotechnology

Metabolic traits ruling the specificity of the immune response in different cancer types

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Cancer immunotherapy aims to augment the response of the patient's own immune system against cancer cells. Despite effective for some patients and some cancer types, the therapeutic efficacy of this treatment is limited by the composition of the tumor microenvironment (TME), which is not well-suited for the fitness of anti-tumoral immune cells. However, the TME differs between cancer types and tissues, thus complicating the possibility of the development of therapies that would be effective in a large range of patients. A possible scenario is that each type of cancer cell, granted by its own mutations and reminiscent of the functions of the tissue of origin, has a specific metabolism that will impinge on the metabolic composition of the TME, which in turn specifically affects T cell fitness. Therefore, targeting cancer or T cell metabolism could increase the efficacy and specificity of existing immunotherapies, improving disease outcome and minimizing adverse reactions.

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Current Opinion in Biotechnology 2021, **68**:124–143

This review comes from a themed issue on **Systems biology**

Edited by **Karsten Hiller** and **Dirk Brenner**

https://doi.org/10.1016/j.copbio.2020.10.011

Introduction

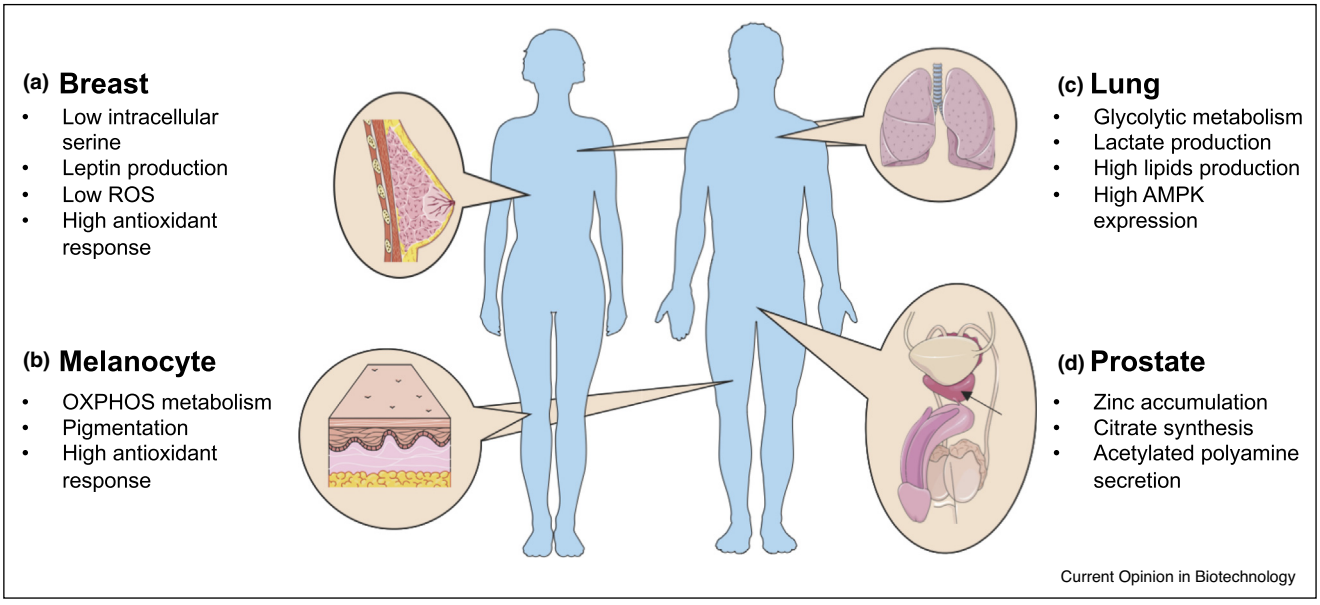
Immunotherapy is a promising treatment option for patients with different cancers resistant to conventional therapies. Therapeutic regimens such as adoptive T cell transfer (ACT), cancer vaccines and immune checkpoint inhibitors (ICI) (e.g. α-PD-1, α-PDL-1 or α-CTLA-4 antibodies), harness the ability of the immune system to recognize and reject the tumor [1]. T cells are the major players in these therapeutic options due to their antigen specificity, robust killing capacity and longevity. Despite these intrinsic T cell characteristics, immunotherapy has been largely ineffective for the majority of patients. It is believed that this low efficacy is due to the highly immunosuppressive tumor microenvironment (TME) which limits the infiltration of the invigorated immune cells delivered by immunotherapy. Cancer cell metabolism contributes greatly to this immunosuppressive environment by depleting nutrients and secreting metabolites that inhibit anti-tumoral effector T cells [2].

Cancer cells are well known for their ability to modulate their metabolic processes in order to fuel their uncontrolled proliferation and create an environment which prohibits the infiltration of effector T cells while recruiting immunosuppressive cell types such as regulatory T cells (Treg) or tumor-associated macrophages (TAMs) [3]. Research suggests that the metabolic reprograming of cancer cells is one of the major factors responsible for the development of therapy resistance [4]. Therefore, targeting metabolic processes of cancer cells and/or T cells in combination with immunotherapy could boost the efficacy of this therapeutic option.

An additional parameter to consider when designing therapeutics targeting cancer-metabolism or immunometabolism is the organ in which the tumor develops. Different tissues support different cellular functions which are accompanied by distinct metabolic needs. An example of tissue with a very specific cellular metabolism is the prostate. Epithelial cells in the prostate are well characterized to produce citrate instead of oxidizing it in the TCA cycle as it is common in other tissues (Figure 1) [5]. Furthermore, different oncogenic addictions or oncosuppressive cues (recurrent in some but not in other tumor histotypes) can rewire the metabolism of cancer cells towards a specific direction. For example, copy numbers of oncogenic KRAS mutant in lung cancer (LC) correlate with augmented glucose uptake and increased channeling of glucose-derived metabolites into the TCA cycle [6]. These two considerations lead to the obvious conclusion that cancer types originating in the same organ can be characterized by very different metabolic profiles requiring a different treatment approach (Box 1).

The presence of tumor infiltrating lymphocytes (TILs) in solid tumors is strongly correlated to improved disease outcome. T cells represent the major cell type in TILs. Of those, $CD8^+$ T cells most strongly correlate with favorable

Figure 1



Metabolic features in healthy tissue. **(a)** Metabolism in healthy breast tissue is characterized by low concentrations of intracellular serine, high production and secretion of the adipokine leptin. Additionally, breast tissue has low amounts of ROS due in part by high antioxidant response. **(b)** Melanocytes in healthy skin have a low proliferative rate and metabolism that is dominated by OXPHOS. High antioxidant response and melanin pigmentation protect these cells from UV radiation and ROS. **(c)** Lung epithelium relies mainly on a glycolytic metabolism and produces high levels of lactate even in presence of oxygen. Moreover, lung epithelium is characterized by high lipids synthesis and expression of the metabolism sensor AMPK. **(d)** Prostate epithelium is known for accumulation of Zinc and high citrate synthesis as well as acetylated polyamine secretion.

clinical results and are therefore considered essential for the fight against cancer. On the other hand, Treg cells contribute to the immunosuppressive TME and inhibit anti-tumor T cell function. Unsurprisingly, in most cases the presence of Treg cells in the tumor has a negative effect on patients' disease outcome. Although T cells are undoubtedly important players in the body's immune response against all cancer types, this response has been shown to vary between different cancers. Considering the great metabolic heterogeneity between cancer types, and even between cancers with the same origin, it is reasonable to consider that differences in T cell response are a direct consequence of these metabolic characteristics. Furthermore, generally, effector T cells share many of the same metabolic features as most cancer cells (such as increased aerobic glycolysis and anabolic metabolism). This leads to metabolic competition between these cells which ultimately impacts disease progression. For example, cancer cells deplete glucose in the TME at the expense of T cells therefore inhibiting their anti-cancer functions [7].

In this review we discuss the link between cancer cell metabolism and T cell response using examples from the most recent research done on melanoma, lung, breast and prostate cancers which are some of the most common primary cancer sites. Recently described possible metabolic targets for the treatment of these cancers are outlined in Table 1.

Melanoma

Melanoma is one of the most aggressive cancers due to its ability to disseminate from a small primary tumor. According to the American Cancer Society there will be over 100 000 cases of melanoma diagnosed in the US in 2020 making it the cancer type with the fifth highest incidence[8]. Melanoma is a highly genetic heterogenous type of tumor. The most common oncogenic mutation is BRAF (in about 45–50% of melanoma tumors), followed by NRAS (30%) and NF1 (10–15%), all genes involved in the mitogen-activated protein kinase (MAPK) pathway, while the remaining 5–10% of melanoma are considered as triple wild-type tumors (TWT) and are driven by other mutations. In physiological conditions, melanocytes are quiescent cells with a low proliferative rate. Their main role is to produce and transfer melanin to the keratinocytes in order to protect the skin against UV radiations. In healthy tissue, melanocytes rely heavily on oxidative phosphorylation and are able to survive reactive oxygen species (ROS) and UV-induce damage thanks to melanin and a good antioxidant response (Figure 1).

Box 1 Main conclusions and future impact of tissue and tumor specificity of (immune)metabolism in the treatment of different types of cancer:

Immunotherapy represents a significant breakthrough in modern medicine. It has improved patients' lives in many different disease areas, from autoimmune disorders such as asthma and atopic dermatitis where it contributes to better quality of life, to cancer where in some cancer types it can contribute to cancer cure and extended lifespan. Despite its great success in a subset of cancer types, immunotherapy is still largely ineffective in many solid tumor types and in large patients' subsets. In the quest for a reason why immunotherapy is effective in some cancer types (belonging to a specific histotype or carrying a defined genetic background or mutations), but not in others, scientists have turned to cellular metabolism for answers. General characteristics of cancer cell metabolism are well described, however depending on the organ of origin and some recurrent mutations in oncogenes or oncosuppressors, cancer cell metabolism can vary significantly. This is greatly influenced by general organ metabolism and/or activation of metabolic pathways/dependency that are downstream the genetic alterations. Consequently, cancer cell metabolism influences the infiltration and the activity of immune cells at the primary tumor site. Research is still lacking on the connection between cancer cell and immune cells metabolism. Considering T cells are the main targets of immunotherapy, they are the obvious choice of cell types to focus on. What is also obvious is that one solution will not work for all cancer types, therefore we must study metabolic characteristics and identify targets, in both T cells or cancer cells, that could be exploited in order to boost immune infiltration and response within different types of solid tumors. This kind of approach would likely be useful in combination with conventional immunotherapeutics such as immune checkpoint inhibitors or adoptive cell transfer.

The recent research on immune- and cancer cell metabolism in different cancer types, with different organ of origin, presented here points to different metabolic pathways as promising new directions for cancer therapy. For example, in melanoma, recent findings demonstrate that Inosine supplementation *in vivo* can increase the efficacy of anti-PD-1 and ACT treatments [34••] as well as points to methionine as a nutrient specific for $CD8^+$ T cells functionality [35••]. In lung cancer, new report presents evidence of the role of deacetylase Sirtuin-2 (Sirt2) as a metabolic immune checkpoint, which negatively regulates key enzyme of glycolysis and oxidative phosphorylation in CTLs [54••]. In breast cancer, it was shown that targeting the leptin-STAT3-FAO pathway results in enhanced anti tumoral $CD8^+$ T cell response in addition to inhibiting cancer cells [77•]. In prostate cancer, a recent report suggests the potential of a therapeutic effect of IDO inhibition in combination with different types of therapies, such as with other metabolic targets in addition to immunotherapy [96].

Currently much of the research is done in a small subset of cancer models, partly due to the ease of manipulation in some animal models and the availability of well described cancer cell lines. However, it is clear that the research done on one cancer type is not necessarily applicable to other cancer types, or even subtypes within the same broader cancer type category. Though more time consuming, spontaneously occurring tumors (enforced by exposure to carcinogenic agents or diet) might resemble some of the aspects that are seen in humans, to a better extent than grafted cancer cells (even when orthotopic) or oncogene-driven genetically engineered mouse models (GEMMs). Therefore, more research focusing on tumors with different organs of origins that resemble more closely what's happening in 'cancer evolution' is urgently necessary in order to determine the most effective therapeutic for patients inflicted by this deadly and heterogeneous disease.

Melanoma cell metabolism

Triggering of the MAPK pathway in melanoma cancer cells leads to an upregulation of gene expression related to glycolysis even in rather normoxic conditions, resulting in an aggressive phenotype. In BRAF-mutated melanomas, the MAPK-activated p90 ribosomal S6 kinase (RSK) directly phosphorylates PFKFB2, an enzyme involved in the glycolysis pathway. Phosphorylation-deficient mutant of PFKFB2 decreased aerobic glycolysis and reduced tumor growth in mouse models of melanoma [9]. Hence, it is not surprising that the most common therapies for the treatment of melanoma patients, namely the inhibitors of BRAF or MEK (a downstream regulator of MAPK), have an even greater beneficial effect when combined and this effect can be partly due to a block in cell metabolism (and glycolysis). This and other melanoma specific metabolic characteristics are depicted in Figure 2.

However, there is still a large fraction of patients that relapse or become therapy resistant. Recent papers have linked the resistant phenotype of melanoma tumors with a subset of cancer cells characterized by an enhanced oxidative metabolism. Melanoma cells resistant to MAPK inhibitors develop an oxidative phenotype, relying more on glutamine consumption and oxidation [10]. Moreover, the upregulation of ATP-Citrate Lyase (ACLY) specifically activates, through epigenetic regulation, the MITF–PGC1α axis to promote mitochondrial biogenesis and melanoma growth. Interestingly, the combination of MAPK and ACLY inhibition was able to reduce tumor growth [11].

In addition, Gabra *et al.* showed that dietary glutamine supplementation reduces tumor growth and improves the response to BRAF inhibitors. Specifically, it leads to increased intra-tumoral α-ketoglutarate concentration that drives hypomethylation of H3K4me3, thereby suppressing epigenetically activated oncogenic pathways in melanoma [12]

The most relevant cause of morbidity and mortality in melanoma is metastatic disease. Therefore, a major research focus is on targeting the metastatic spread of melanoma cells. The accumulation of lactate in the tumor microenvironment (TME), consequent to the increased glycolytic flux has an important role in metastasis formation. Analysis of patient-derived xenografts and syngeneic models revealed that cells with the greatest metastatic potential were able to utilize lactate as a carbon source, through the upregulation of monocarboxylate transporter 1 (MCT1). Consistently, the inhibition of this transporter had little effect on tumor growth but induced a strong impairment on metastasis formation [13•]. Other findings linked AMP-activated Protein Kinase (AMPK) activation in metastatic lesions with an increased oxidative phosphorylation (OXPHOS) and glutamine consumption. Consequently, these cells showed a higher resistance to

Table 1

Potential metabolic targets for the treatment of melanoma, lung, breast and prostate cancer

Cancer type	Cancer subtype	Potential Metabolic Target	Role in disease	Pharmacological inhibitor	Clinical observations	Proposed therapeutic setting	Reference
Melanoma	Braf mutated	RSK	Inhibition of RSK decreases the metabolic flux in cancer cell reducing tumor growth	LJH685	None	Unknown	[9]
		ACLY	Inhibition of ACLY sensitized to MAPK inhibition by suppressing MITF–PGC1a axis.	HC and SB-204990	None	In combination with MAPK inhibitor	[11]
	GRM1+	glutamate	Inhibition of GLS and glutamate release promotes apoptotic cell death	CB-839 + riluzole	None	Unknown	[18]
	MAPKi resistant	OXPHOS	Inhibition of OXPHOS metabolism reduce the number of brain metastasis and improve mice survival	IACS-010759	None	Unknown	[15]
		OXPHOS	Inhibition of oxidative metabolism in cancer cells improve T cells activity and response to α-PD-1		None	In combination with α-PD-1	[21•]
		LDHA (cancer cells)	Inhibition of cancer cells glycolysis promotes T cell-mediated apoptosis	GSK2837808A	None	In combination with ACT	[19••]
		LDHA (T cells)	Pretreatment of T cell with LDHi and IL-21 promotes stem cell memory T cells formation and improves ACT	NCI-737	None	In combination with ACT	[26]
		MCT1/ MCT4	Inhibition of lactate transport restricted cancer cells glycolysis and improves T cells function	Diclofenac	None	In combination with α-PD-1 and CTLA-4	[20]
		MCT1	Inhibition of MCT1 on cancer cell does not affect primary tumor growth but reduces the number of metastasis	AZD3965	None	Unknown	[13•]
	Unspecified	Enolase 1	Adding pyruvate restore glycolytic and oxidative metabolism of $CD8^+$ T cell improving their effector functions	pyruvate	None	Unknown	[25••]
		FATP1/ SLC7A1	FATP1 inhibition decreases fatty acid uptake in cancer cells and reduces melanoma growth and invasion	Lipofermata/CB16.2	None	Unknown	[16]
		CD36	Blocking of CD36 impaired T_{reg} and reinforces anti-tumor immunity	α-CD36 monoclonal antibody	None	Alone or in combination with α-PD-1	[29••]
		Glutamine metabolism	Glutamine antagonism suppress oxidative and glycolytic metabolism in cancer cells, while promotes T cell anti-tumor functions	JHU083	None	Alone or in combination with ACT or α-PD-1	[33••]

Table 1 (*Continued*)

Cancer type	Cancer subtype	Potential Metabolic Target	Role in disease	Pharmacological inhibitor	Clinical observations	Proposed therapeutic setting	Reference
		SphK1	Inhibition of the SphK1/ PPARγ axis in T cells improves T cell mediated tumor response	PF543	None	In combination with α-PD-1	[32•]
		UCP2	Overexpression of UCP2 in cancer cells increase $CD8^+$ T cell and dendritic cell anti-tumor immunity	rosiglitazone	None	In combination with α-PD-1	[23]
		PPAR-α	Stimulation of PPAR-α increases FAO and improves TILs functionality	Fenofibrate (PPAR-α agonist)	None	In combination with α-PD-1	[27•]
		GCLC	Deletion of Gclc in Treg cells reduces GSH production and disrupt Treg functionality	buthionine sulfoximine	None	Unknown	[31]
			Glutamine dietary supplementation increases glutamine and its downstream metabolite αKG driving hypomethylation of H3K4me3	Glutamine supplementation	None	Alone or in combination with Braf inhibitor	[12]
		ROS	Cell-permeant form of cysteine increases glutathione synthesis, therefore reduces ROS and restore self-renewal in exhausted T cells	N-acetylcysteine	None	In combination with ACT	[28]
			Inosine supports the function of Teff cells in the absence of glucose	Inosine supplementation	None	In combination with α-PD-1 and ACT	[34••]
		SLC43A2	Its inhibition increases methionine availability for the T cells resulting in increased dimethylation of H3K79me2 and improved T cell immunity.	Methionine supplementation or BCH	SLC43A2 is hihgly expressed in cancer cells compared to T cells and normal tissue. It correlates with poor survival	In combination with αi-PDL-1	[35••]
Lung		GLDC	GLDC inhibition impairs pyruvate metabolism, inducing tumor regression	GLDC-shAON	None	Alone or in combination with cisplatin	[37]
		PRODH	PRODH inhibition reduces tumor growth, block epithelial to mesenchymal transition and inhibits inflammatory cytokines release such as CXCL1, LCN2 and IL17C	L-THFA	PRODH highly expressed in AdC tumors compared to normal tissue	Unknown	[48]
	NSCLC	Malin	Downregulation of malin induces nuclear glycogen accumulation and decrease histone acetylation, thus promoting tumor growth	None	High malin mRNA expression correlates with better survival	Unknown	[50•]
		IGF-1R	Inhibition or downregulation of IGF-1R synergize with PD-1 inhibition to boost CD8 + T cells, leading to reduced tumor growth	PQ401	High IGF-1 plasma levels/ high IGF-R1 tumor expression is associated with resistance to α-PD-L1 immunotherapy	In combination with α-PD-L1	[55••]
	NSCLC AdC	MGST1	MGST1 knockdown promotes cancer cells apoptosis and suppressed tumor growth *in vivo*	None	High expressed in human AdC. The expression correlates with poor overall survival.	Unknown	[38]

Table 1 (*Continued*)

Cancer type	Cancer subtype	Potential Metabolic Target	Role in disease	Pharmacological inhibitor	Clinical observations	Proposed therapeutic setting	Reference
	NSCLC SCC	GLS	GLS inhibition overcomes resistance to mTOR inhibition	CB-839	None	In combination with mTOR inhibitor (MLN128)	[47••]
	NSCLC EGFR mutant	JNK/ Glucose metabolism	JNK direct activation or indirect activation through inhibition of glucose metabolism, induces EGFR-mutant NSCLC cells apoptosis and overcome resistance to tyrosine kinase inhibitors.	Anisomycin/ 2DG	Expression of phosphorylated JNK is significantly decreased in EGFR mutant NSCLC compared to EGRF WT	Unknown	[39]
	NSCLS KRAS mutant	KRAS	KRAS inhibition induces an inflamed tumor microenvironment, leading to tumor regression	AMG 510	In clinical trial (NCT03600883) induced objective partial response or stable disease	Alone or in combination with carboplatin or immunotherapy	[40••]
		ASNS	Inhibition of ASNS decreases tumor growth	L-asparaginase	None	In combination with AKT inhibitor	[41]
		AMPK	AMPK deletion suppresses tumor growth		None	Unknown	[45]
	NSCLC KRAS mutant KEAP1 deficient	Pentose phosphate pathway	Inhibition of this pathway abrogates tumor growth	6-AN	None	Unknown	[42]
	NSCLC KRAS mutant LKB1 deficient	Atg7	Deletion of Atg7 abrogates both tumor initiation and growth		None	Unknown	[43]
	NSCLC KRAS mutant LKB1 deficient KEAP1 mutant	GLS	This set of mutations promote a glutamine-addictive metabolism, rendering the tumor sensitive to glutaminase inhibition	CB-839	None	Unknown	[44]
	NSCLC SMARCA4 mutant	OXPHOS	Mutations in the SWI/SNF complex induce dependence on OXPHOS, sensitizing tumors to its inhibition	IACS-010759	High expression of OXPHOS-related genes in SMARCA4 mutant patients	Unknown	[49•]
		DHODH	DHODH inhibition attenuates primary tumor growth and delays liver metastasis	Brequinar	None	Alone or in combination with cisplatin/ etoposide	[52••]
	SCLC	MEK5/ ERK5	Depletion of MEK5/ERK5 pathway perturbs different lipid metabolism pathways and sensitizes SCLC cells to mevalonate inhibition		None	Unknown	[53]
Breast	TNBC	Cholesterol synthesis pathway enzymes	Inhibition of this pathway leads to a reduction of TNBC propagation	Simvastatin (inhibitor of the key enzyme in the cholesterol biosynthesis pathway, 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase)	None	Unknown	[66•]
		Estrogen-related receptor α (ERRα)		Cpd29	None	In combination with GLS1 and G6PD inhibitors	[69]

Table 1 (*Continued*)

Cancer type	Cancer subtype	Potential Metabolic Target	Role in disease	Pharmacological inhibitor	Clinical observations	Proposed therapeutic setting	Reference
	ER- and HER+	AKR1B10	promotes metastasis by limiting the oxidative stress and enabling fatty acid oxidation in the metastatic environment	None	None	Unknown	[70••]
	Obesity promoted BC	STAT3	Deletion of STAT3 leads to increased $CD8^+$ T cell effector function	None	None	Unknown	[77•]
	Not specified	Atg5	Deletion of Atg5 results in increased glycolysis in $CD8^+$ T cells and a shift to a memory phenotype resulting in decreased tumor burden in e0771 mouse BC model.	None	None	Suggested use combination with immunotherapy	[82••]
Prostate		IDO	Increased IDO inhibits effector T cell functions. IDO expression could be a mechanism of immune evasion is prostate cancer	Several described (Epacadostat, Navoximod..)	Increased IDO activity in patients after immunotherapy	In combination with immunotherapy	[95]
		Hypoxia	Hypoxic regions of tumors are characterized with absence of T cell infiltration.	hypoxia activated pro-drug TH-302	None	In combination with immunotherapy	[93•]
		Polyamine expression and MTAP	Increased polyamine expression together with the inhibition of MTAP.	BENSpm (polyamine analog to boost acetylated polyamine secretion) and MTDIA (inhibitor of MTAP)	None	Suggested use together with Docetaxel	[89••]
		SIRT7	SIRT7 activation inhibits glycolysis and leads to the upregulation of FAO. Deletion of SIRT7 leads to increased $CD8^+$ T cell effector functions.	ID: 97491[100]	None	Unknown	[91•]
		DRP1	DRP1 Expression promotes cancer cell survival under metabolic stress conditions	None	None	Unknown	[90]

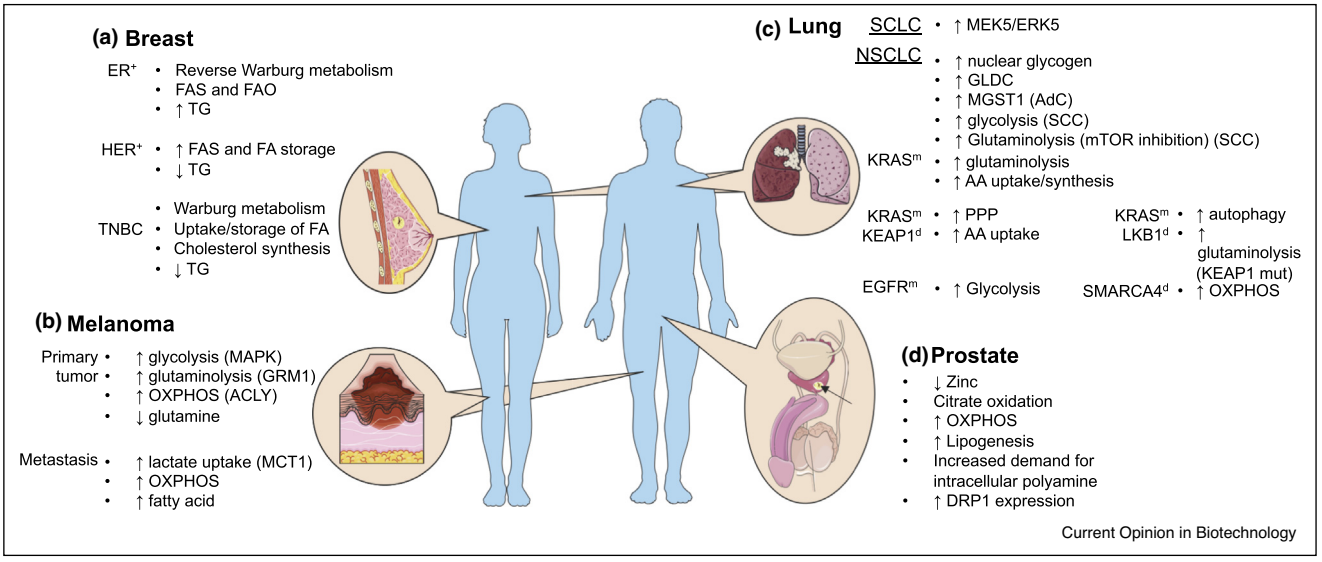
oxidative stress and, therefore, a better survival [14]. Moreover, a transcriptomic analysis of brain metastasis compared with patient-paired extracranial metastasis revealed an increase in OXPHOS in brain lesions. Consistently, inhibition of OXPHOS in murine models reduces the number of metastasis in the brain but not in the lungs [15].

Upon metastatic disease initiation, melanoma cancer cells initiate their cross-talk with the adipocytes present in the subcutaneous region [16]. Adipocytes transfer lipids via FATP1/SLC7A1 transporter overexpressed in melanoma cells, boosting cancer cell proliferation and invasion [16]. Fatty acids are also important in the case of *Mift*-mutated melanomas, which are part of the TWT group, where the reduction of this transcription factor decreases the level of fatty acid saturation, impacting on cancer cell proliferation [17].

In addition to fatty acids and glucose, glutamate also plays an important role in melanoma. Hyperactivation of glutamate metabotropic receptor (GRM1) in malignant melanoma is an oncogenic driver and GRM1- activated melanomas were found to exhibit significantly increased expression levels of glutaminase (GLS). In an *in vivo* model of human melanoma cells engrafted in immunodeficient mice, concurrent inhibition of glutaminolysis and glutamate release was shown to suppress tumor progression [18].

To conclude, although glycolysis and its products have a strong impact on melanoma tumor aggressiveness and progression, recent findings highlighted the importance of other metabolic pathways, like fatty acid oxidations or glutaminolysis, that can considerably affect cancer cells proliferation and therefore tumor progression.

Figure 2

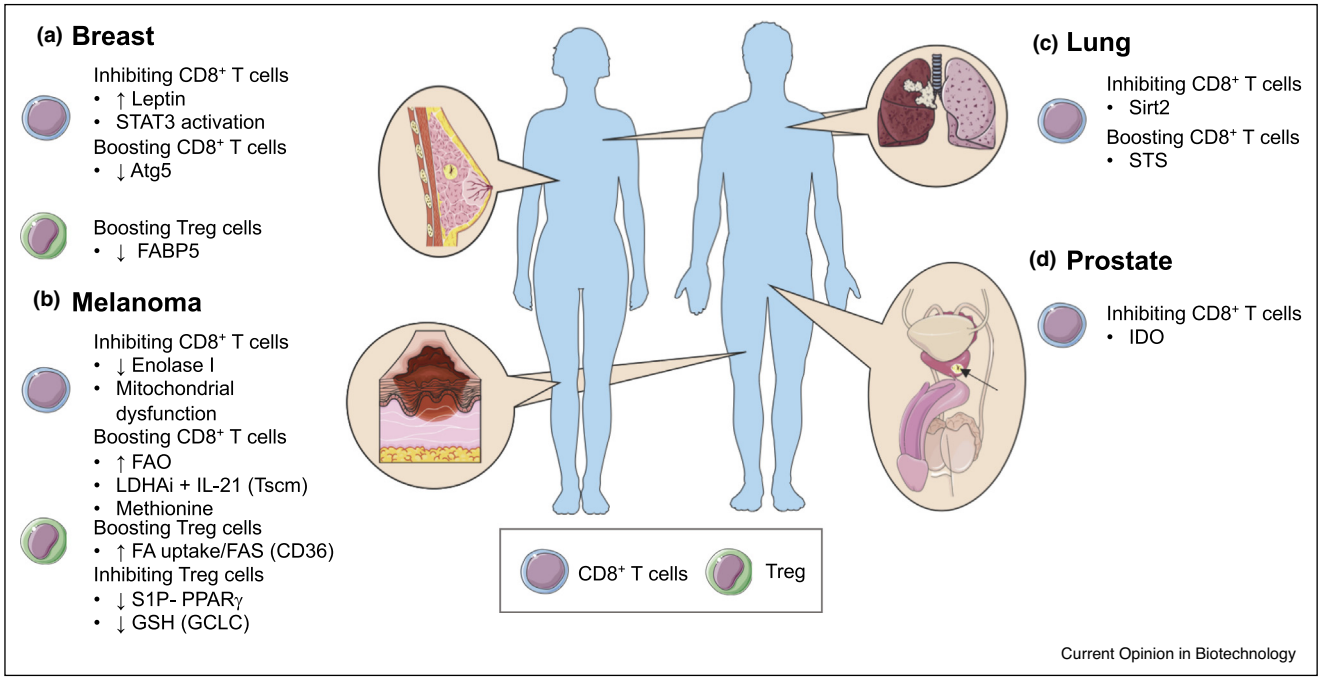


Tissue specific cancer metabolism. **(a)** Breast cancer specific metabolism. ER + BC subtype is characterized by Reverse Warburg metabolism – influencing neighboring stromal cells to upregulate OXPHOS and channel metabolites to support cancer cells. Regarding fatty acids, ER + BC prefers FAS and FAO while TNBC relies more on uptake and storage of exogenous fatty acids. HER + BC relies heavily on FAS and has high rates of fatty acid storage. Additionally, TNBC exhibits classical Warburg metabolism and is also dependent on cholesterol synthesis. ER + BC has upregulated triacylglycerols (TG), while TNBC and HER + BC is known to downregulate TG. **(b)** Melanoma metabolism. Melanoma primary tumors have increased glycolysis (mainly driven by MAPK), increased glutaminolysis (due to hyperactivation of GRM1) and augmented oxidative phosphorylation epigenetically potentiates by ACLY. Moreover, dietary glutamine supplementation has been showed to reduce tumor growth. Melanoma metastases have been shown to have increased lactate uptake due to the upregulation of MCT1, augmented OXPHOS and increased fatty acid metabolism. **(c)** Lung cancer metabolism. SCLC is characterized by an increased MEK5/ERK5 kinases activity which promotes proliferation and controls lipid metabolism. NSCLC has been shown to accumulate glycogen in the nucleus and express higher GLDC, mechanisms that rewire cancer metabolism and sustain its growth. Different subtypes harbor also metabolic differences and in particular, in adenocarcinoma (AdC) higher expression of MGST1 has been detected and associated with poor prognosis, whereas squamous cell carcinomas (SCC) are characterized by higher glycolysis and mTOR activity, inhibition of which leads to a preferential glutamine metabolism. Oncogenic mutations are often found in NSCLC, inducing different metabolic pathways. KRAS activating mutations induce glutamine consumption and increased amino acids uptake and synthesis. When KRAS mutations are accompanied by KEAP1 loss of function, cancer cells show a higher PPP and AA uptake, whereas, in presence of LKB1 loss of function, they show an increased autophagy to sustain an elevated macromolecular biosynthesis. Additionally, in KRAS mutant, LKB1 deficient NSCLC, activating mutation of KEAP1, induce higher glutamine consumption. Moreover, activating mutations in EGFR promote a glycolytic addictive metabolism, whereas loss of function mutations in SMARCA4 promote dependency on OXPHOS. **(d)** Prostate cancer metabolism. Malignant prostate cells convert to citrate oxidation and a decrease in Zinc concentrations, together with increased demand for intracellular polyamines. Androgen receptor signaling in prostate cells upregulates expression of DRP1 which leads to increased OXPHOS and lipogenesis.

T cell metabolism in melanoma

Together with BRAF and MEK inhibitors, immunotherapy has become an important treatment option for melanoma patients. The discoveries of immunotherapy and in particular ICI, had a strong impact on the survival of melanoma patients. However, there is still a large number of patients that do not benefit from this treatment. In melanoma, T cells constitute a high percentage of the immune infiltrate, but they often retain a pro-tumoral or exhausted phenotype. Recent advances have shown how targeting melanoma cell metabolism can change the TME, improving the fitness of the endogenous T cells and, therefore, the efficacy of immunotherapies (Figure 3). Cascone *et al.*, for example, have linked the augmented glycolysis of a fraction of cancer cells with the resistance to ACT in melanoma patients [19••]. Glycolysis-related protein, in fact, are among the most studied targets in combinational therapies with the ultimate goal to improve the outcome of immunotherapy treatments. Consistently, restricting glycolysis using diclofenac, as a non-specific inhibitor of both lactate transporters MCT1 and MCT4, reduces cancer cell proliferation preventing them from using lactate as a carbon source. This inhibitor affects also T cell metabolism by decreasing their proliferation, but not their activation or cytokine production. Consequently, diclofenac treatment increases the response to α-PD-1, improving mice survival [20]. Moreover, Najjar *et al.* compared an array of melanoma cell lines characterized by glycolytic or oxidative metabolism. They demonstrate that a low oxidative metabolism results in a less hypoxic microenvironment that helps the infiltration and activation of $CD8^+$ T cells,

Figure 3



Metabolic factors influencing T cell responses in different tumor types. **(a)** T cell metabolism in response to breast cancer. Leptin expressed by mammary adipocytes activates STAT3 in CD8+ T cells, which induces FAO and inhibits glycolysis leading to decreased effector function. Deletion of Atg5 in T cells results in a shift to a memory phenotype and an increase of proinflammatory cytokine expression. Inhibition of FABP5 leads to increased Treg suppressive function. **(b)** T cell metabolism in melanoma. Compromised activity of Enolase I leads to impaired glycolysis and oxidative metabolism and reduced effector cell function. Exhausted T cells present mitochondrial dysfunction. FAO and methionine are necessary to maintain TIL functionality. LDHA inhibition together with IL-21 treatment of T cells before ACT results in increased Tscm polarization and a more effective anti-tumoral response. CD36 enhances lipid uptake in Treg cells. Targeting the S1P-PPARγ pathway or GSH production mediated by GCLC decreases Treg polarization and function. **(c)** T cell metabolism in Lung cancer. Sirt-2 acts as a metabolic immune checkpoint. Its inhibition enhances T cells metabolic fitness and effector function. Short term starvation (STS) increases CD8/Treg ratio and synergizes with anti-PD-L1 treatment. **(d)** T cell metabolism in prostate cancer. IDO expression leads to a decrease in tryptophan and subsequently in the inhibition of effector T cell function.

ultimately reducing tumor growth and improving α-PD-1 response [21•]. In contrast with these findings, a proteomic analysis of different cohorts of melanoma patients treated with α-PD-1 or ACT, highlighted an increased lipid and oxidative metabolism in patients that responded better to the treatment. Additionally, augmented mitochondrial metabolism was linked with a higher antigen presentation and IFNγ signaling [22•]. Accordingly, deletion of Acetyl-CoA acetyltransferase to impede oxidative metabolism, strongly reduced MHC class 1 expression and increased tumor growth [22•].

Independently from glycolysis, another study shows how Uncoupling protein 2 (UCP2) expression in melanoma patients correlates with T cell infiltration and prolonged survival rate. Its overexpression in cancer cells was found to change the cytokines composition of the TME, increasing CD8+ T cell infiltration in a dendritic cells-dependent manner and finally sensitizing tumors to PD-1 treatment [23]. This effect seems to be independent from the uncoupling function of UCP2, but the underlying mechanism is still unclear.

Gut microbiota composition can also influence the response to ICI. Analysis of serum and stool microbiota of two different cohorts of melanoma patients revealed an association between the two short chain fatty acids (SCNA) butyrate and propionate with clinical outcomes. A high concentration of these SCNA correlates with resistance to α-CTLA4. In mice, treatment with butyrate reduces the efficacy of α-CTLA-4 treatment leading to a decrease in CD80 and CD86 expression on the surface of dendritic cells and a decrease in memory T cells number [24].

On the other hand, targeting the metabolism of T cells can also have a strong impact on their function and polarization. TILs in human melanoma display an impairment of both glycolytic and oxidative metabolism, due to the compromised activity of enolase I, an

important glycolytic enzyme. Ultimately, restoration of its function improves both glycolytic and oxidative metabolism, boosting effector T cell functions [25••]. Interestingly, a transcriptomic analysis aimed to analyze the different effects of IL-2 and IL-21 on T cell metabolism, highlighted LDHA as one of the most differently expressed genes. IL-2 treatment increases T cell glycolytic flux and polarizes them towards an effector phenotype, whereas IL-21 maintains a more quiescent metabolism and promotes stem cell memory T cells (Tscm). Accordingly, LDHA inhibition combined with IL-21 treatment of T cells before ACT, increases the formation of Tscm cells, resulting in a more profound anti-tumor responses and a prolonged host survival [26]. In addition, $CD8^+$ T cells isolated from murine and human melanoma tumors demonstrate an increased fatty acid uptake and oxidation [27•]. In particular, catabolism of fatty acids is necessary to maintain TIL functionality [27•]. Another study showed how exhausted T cells have defect in oxidative phosphorylation. An antioxidant treatment was enough to restore T cells functions [28].

Treg cells infiltrating different types of tumors, including melanoma, have an enhanced lipid uptake mediated by the transporter CD36, which also fuels oxidative mitochondrial fitness via PPAR-β. Accordingly, genetic deletion or pharmacological targeting of CD36 decrease Treg number in the tumor, create a less suppressive TME and synergize with α-PD-1, thus leading to a reduced tumor growth [29••]. Interestingly, not only fatty acid uptake is important for Treg cells proliferation, but also FAS. Both glycolytic and oxidative pathways are necessary to fuel FAS and allow Treg expansion in the TME. The ability of this cell type to utilize different metabolic routes confers them a growth advantage as compared to conventional T cells [30].

Moreover, Treg cells display a highly antioxidant phenotype mediated by reduced glutathione (GSH). Specific deletion of *Gclc*, a subunit of the glutamate cysteine ligase involved in GSH synthesis, revealed how targeting GSH production in Treg cells reduces serine metabolism and impacts on the functionality of this T cell subset. *In vivo Gclc* deletion induces a strong autoimmune effect and improves anti-tumor immunity. In particular, ACT of Gclc depleted Treg cells together with T effector (Teff) cells had a greater anti-tumoral activity compared to ACT with wild-type Treg and Teff cells, due to their reduced immunosuppressive effect [31].

Furthermore, it is known that Sphingosine 1-phosphate (S1P) controls T cell egression from lymphoid organs and Treg and Th17 cell polarization. Chakraborty *et al.* demonstrated that T cells lacking sphingosine kinase 1 (SphK1), the enzyme involved in the production of S1P, maintain T central memory phenotype and decrease their polarization towards Treg cells. They proved that these cells exhibit an increased OXPHOS and lipolysis mediated by PPARγ and that targeting the S1P- PPARγ axis improves the anti-tumor response [32•].

Finally, inhibition of glutamine metabolism by the use of a pro-drug of 6-diazo-5-oxo-L-norleucine (DON), a glutamine antagonist that becomes active in the TME upon enzymatic cleavage, has a strong impact on the overall metabolism of melanoma cells and affects their proliferation. Whereas the same molecule has the opposite effect on T cells by increasing their proliferation and reducing exhaustion and anergy. T cells, differently from cancer cells, are able to utilize acetate as carbon source to fuel the TCA, thanks to the up-regulation of acyl–coenzyme A synthetase short-chain family member 1 (ACSS1). Therefore, the use of this inhibitor in mouse models shows a strong decrease in tumor growth and a synergistic effect with α-PD1 treatment [33••].

Moreover, Teff cells are able to utilize inosine as an alternative energy source when glucose is missing, fueling their proliferation and effector functions. On the contrary different cancer cell lines seem to be unable to use the same metabolite. Consistently, inosine supplementation *in vivo* increases the efficacy α-PD-1 and ACT treatments [34••].

An alternative study highlights instead the importance of methionine as a nutrient specific for CD8 T cells functionality. The deprivation of methionine in the TME caused by its highly consumption from cancer cells decreases dimethylation at lysine 79 of histone H3 (H3K79me2) in T cells, leading to a low expression of STAT5 and impaired T cell immunity. Consistently, pharmacological or genetical inhibition of SLC43A2 in the tumor improves response to immune-checkpoint therapy [35••].

In conclusion, recent work brought to attention the heterogeneity of melanoma metabolism highlighting the effect of cancer cell metabolism on T cell functionality and vice versa. In addition, these last findings underline the importance of studying metabolic vulnerabilities specific to cancer cells, in order to open new possibilities for the creation of specific therapies targeting cancer cells while at the same time promoting anti-tumor immune cell functions. While contradictory results leave space for further study regarding the contribution of oxidative metabolism to immunotherapy resistance, growing evidences indicate the importance of targeting glycolysis and to further study the role of fatty acids in the polarization and functionality of T cells, in particular of Treg cells, opening the way for new possible treatments.

Lung

LC displays the highest incidence worldwide (2 million in 2018) as well as being the leading cause of cancer related

deaths worldwide (1.7 million in 2018), due to a combination of late diagnosis and poor treatment options. This tumor type is highly heterogeneous and categorized into two main groups: Non-Small Cell Lung Cancer (NSCLC), which represents 80–85% of LC patients, and Small Cell Lung Cancer (SCLC), that affects the neuroendocrine tissue of the lungs and occurs in 15–20% of the cases. Based on the histology NSCLC is further subcategorized into Adenocarcinoma (AdC), Squamous cell carcinoma (SCC) and Large cell carcinoma (LCC). Smoking is the first risk factor for LC, and is the cause of 80–90% of the cases. This holds true particularly for SCLC, that develops almost exclusively in heavy smokers, and for the SCC subtype among the NSCLC. In healthy conditions, lung epithelium forms a conduit for gas exchange, but it is also engaged in more specialized energy-consuming activities such as airway clearance, through phagocytosis and ciliary motility, and production of pulmonary surfactant, supported by a high cellular lipid synthesis. For these reasons, lung epithelium is one of the tissues with the highest glucose consumption accompanied by an elevated lactate production, to minimize local oxygen utilization, thus enhancing its delivery to the other tissues. Moreover, energy production and usage are fine-tuned by the metabolic regulator AMPK, highly expressed in this tissue (Figure 1) [36]. LC specific metabolic features are represented in Figure 2.

In 2015 three different drugs, namely nivolumab, pembrolizumab (targeting PD-1), and atezolizumab (targeting PD-L1), received FDA approval for the treatment of advanced stage NSCLC, leading to an improve in survival and in some cases to tumor regression. However, 80% of NSCLC patients do not respond to immunotherapy with some even undergoing hyper progression. Therefore, it is becoming important to identify biomarkers predictive of response or resistance to immune checkpoint therapy, and ways to improve such response or sensitize the tumor to ICI.

Lung cancer metabolism

NSCLC, as many other tumor types, relies on a high glucose and pyruvate consumption to sustain its growth. Among all the glycolytic pathways, glycine decarboxylase (GLDC), which catabolizes glycine to yield 5,10-methylenetetrahydrofolate (MeTHF), is frequently upregulated in various types of cancer including lung, prostate and brain. Particularly in LC, it supports tumor growth, as shown by the fact that GLDC inhibition impairs pyruvate metabolism, thus inducing tumor regression both *in vitro* and *in vivo* [37]. Increased proliferation and mitochondrial activity can be responsible for a higher production of ROS, deleterious products for cell homeostasis. In this context, and differently from what aforementioned in the context of melanomas, Glutathione *S*-transferases (GSTs) are important for cells detoxification. In particular, the microsomal isoform 1 (MGST1) is significantly increased in human AdC, compared to normal lung tissue, and its expression is associated with poor prognosis. Accordingly, MGST1 knockdown, by acting on the mitochondrial apoptotic related proteins, induces apoptosis in AdC cells and suppresses *in vivo* tumor growth [38].

NSCLC is also characterized by a multiplicity of recurrent oncogenic driver mutations, underlining a high variability even among the same histological NSCLC subtype. Different genetic alterations can induce diverse metabolic adaptation and immune response, that can be exploited for therapeutic application. A great example in this sense is provided by the Epidermal Grow Factor Receptor (EGFR), a tyrosine kinase receptor, altered in 15–20% of AdC. EGFR-induced glycolysis sustains cancer proliferation and maintains, at the same time, EGFR stability, thus creating a positive loop [39]. Therefore, the use of Tyrosine Kinase Inhibitors has been effective for the treatment of patients harboring EGFR mutations.

KRAS, a member of the Ras family small GTPases, is involved in the transmission of extracellular mitogenic signals, and its aberrant activation sustains cellular proliferation and survival through RAF-MEK and PI3K/AKT pathways. Therefore, KRAS mutations drive multiple cancers, including LC, where they are found in more than 30% of NSCLC, and mostly in AdC. Despite its high incidence, differently from EGFR, KRAS is difficult to target. Indeed, the first KRAS effective inhibitor is the small molecule AMG510, which was recently proved to induce tumor regression alone and in combination with ICI in a model of colorectal cancer and is now in clinical trial for multiple KRAS-mutant tumors, including NSCLC, for which, based on the preliminary data, it resulted in partial response or stable disease [40••]. In an attempt to find alternative therapeutic strategies to its direct inhibition, the role of mutant KRAS in NSCLC metabolism is being deeply studied. The work of Gwinn D.M. *et al.*, identified KRAS as a key regulator of nutrient deprivation response. Through PIK3-NRF2, KRAS activates ATF4 inducing the expression of its downstream targets, such as glutamine transporter SNAT1 and asparagine synthetase (ASNS), thus regulating amino acid uptake and asparagine biosynthesis [41]. KEAP1, a key player in ROS detoxification, is a negative regulator of NRF2. Interestingly KRAS-driven LC are frequently paired with KEAP1 loss of function mutations. In this context, KEAP1 loss rewires KRAS-mutant tumor metabolism, on one hand by reinforcing ATF4-mediated amino acid uptake and on the other by inducing the pentose phosphate pathway (PPP), inhibition of which abrogates tumor growth [42]. KRAS activation is also commonly coupled with loss of LKB1 function. Patients characterized by KRAS-mutant LKB1-deficient (KL) develop more aggressive tumors, show high frequency of metastasis and are resistant to immunotherapy. Loss of LKB1 sustains KRAS-driven uncontrolled proliferation, even in

a nutrient deprived environment, by promoting macromolecular biosynthesis and inducing autophagy to fuel the reprogrammed metabolism. Consistently, deletion of the autophagy essential gene Atg7 abrogates initiation and growth of KL tumor, suggesting a new therapeutic strategy for this subset of NSCLC [43]. Furthermore, in KRAS-driven AdC, LKB1 loss induces often KEAP1 activation (KLK tumors). By acting cooperatively, this set of mutations maintains energetic and redox homeostasis in a glutamine dependent manner, thus enhancing KLK tumors sensitivity to glutaminase inhibition [44]. LKB1 is also the main positive regulator of AMPK. Interestingly, in a KRAS-mutant mouse model, genetic deletion of AMPK does not phenocopy LKB1 loss and promotes instead tumor regression, pointing out that, in nutrient restrictive conditions, a minimal level of AMPK activity is required for NSCLC growth [45].

SCC are highly aggressive, highly glycolytic and, compared to AdC, show differential expression of a set of genes involved in glucose and glutamine catabolism as well as nucleotide and glutathione biosynthesis [46]. Moreover, SCC, unlike AdC, have frequent mutations in the PIK3/AKT pathway, that result in an overactivation of mTOR and tumor progression. However, single therapies inhibiting this pathway have limited clinical efficacy. As shown by an *in vivo* metabolic and molecular profiling, SCC can adapt to chronic mTOR inhibition and glycolysis suppression via the GSK3α/β pathway, which upregulates glutaminolysis through the induction of GLS, providing a proof of concept for combinatorial GLS and mTOR inhibition [47••].

Epigenetics plays also an important role in metabolic rewiring and tumor progression. In NSCLC the activation of Proline Dehydrogenase (PRODH) mediated by the chromatin remodeling factor LSH, induces tumor growth, epithelial to mesenchymal transition (EMT) and the expression of inflammatory cytokines including CXCL1 and IL17C[48]. Another example is the SWI/SNF chromatin remodeling complex, which is also found altered, mostly in adenocarcinoma. In particular, the chromatin rearrangement mediated by the inactivation of SMARCA4, part of the complex, leads to enhanced oxygen consumption and respiratory capacity. As a result, SMARCA4 mutant xenografts show a dependence on OXPHOS and a marked sensitivity to its inhibition [49•]. Moreover, unlike physiological lung tissue, in NSCLC decreased abundance of malin, an E3 ubiquitin ligase, prevents nuclear translocation of glycogen phosphorylase and induces nuclear accumulation of glycogen, thus impairing histone acetylation and sustaining tumor growth [50•].

Because of the high heterogeneity of LC, it may be important to unveil the relationship between molecular and metabolic cell features on a larger scale. In this sense, an *in vitro* extensive characterization of metabolic fluxes in a high variety of NSCLC cell lines, proved to be useful in predicting dependency on specific metabolic pathways and sensitivity to metabolic drugs such as pemetrexed, an antimetabolite currently in use for NSCLC therapy [51•].

SCLC is frequently characterized by loss of function mutation in tumor suppressor genes TP53 and RB1, but, unlike NSCLC, it rarely presents oncogenic driving mutations, thus limiting therapeutic options. In this regard, through a genetic screening approach, Li *et al.* uncovered SCLC sensitivity toward disruption of the pyrimidine biosynthesis pathway and identified dihydroorotate dehydrogenase (DHODH) as a new promising therapeutic target [52••]. Another pathway that has been studied in SCLC is the MEK5–ERK5 axis. This dual kinase axis is known to be responsible for increased growth and metastasis and lower overall survival in different tumor types including breast, prostate and colon cancer. This is also true for SCLC, where MEK5 and ERK5 play a critical role in cell survival by controlling lipid metabolism. Notably, the loss of MEK5/ERK5 perturbs cholesterol synthesis, making SCLC sensitive to mevalonate inhibitions trough statins [53].

T cell metabolism in lung cancer

An important parameter for the response to ICI seems to be the evaluation of PD-1/PD-L1 expression on both cancer and immune cells, particularly TILs and $CD8^+$ cytotoxic T cells (CTLs). In this context it has been shown that in human NSCLC the presence of $CD8^+$ TILs expressing high levels of PD-1 is predictive of response and survival upon α-PD-1 treatment. Despite the numerous studies analyzing the infiltration of T cells in LC, little is known about their metabolic features in the context of specific nutrients deprived microenvironment. A highly relevant recent discovery points to the role of deacetylase Sirtuin-2 (Sirt2) as a metabolic immune checkpoint, which negatively regulates key enzyme of glycolysis and oxidative phosphorylation in CTLs [54••]. Moreover, the fact that Sirt2 KO can enhance T cells fitness and effector function, leading to an effective antitumor immune response, strongly supports T cells metabolic manipulation as a promising strategy to improve the efficacy of the current immunotherapy regimens [54••].

As a possible way to improve immunotherapy in NSCLC, Ajona *et al.* showed that short term starvation sensitizes tumors to α-PD-1 treatment by reducing circulating insulin-like growth factor 1 (IGF-1) and downregulating IGF-1 receptor (IGF-1R) signaling in tumor cells (Figure 3). Moreover, a combination of IGF-1R inhibition and PD-1 blockade impaired LC progression by boosting intra-tumoral $CD8^+$/Treg ratio, supporting the clinical evaluation of IGF-1 modulators together with α-PD-1 treatment [55••].

SCLC is characterized by a high mutational burden, providing a strong rationale for the use of ICI. Indeed, despite the limited added survival benefit, FDA recently approved the use of atezolizumab (α-PD-L1) together with chemotherapy as first line treatment in extensive stage SCLC (i.e. SCLC spreading far from the primary site, in the lungs themselves or elsewhere). However, like NSCLC, a large number of patients do not respond to the treatment. In this scenario, studies have been conducted to increase T cells infiltration by acting on cancer cells, but manipulation of T cells metabolism has not been exploited so far.

To conclude, an extensive effort has been done to describe the heterogenic lung cancer metabolism. However, T cell metabolic features associated to these different microenvironments are still poorly understood, underling the need for further investigation. In this direction, single cell RNA sequencing aiming to perform large scale NSCLC phenotyping of stromal cells in general or T cells could be useful to improve the outcome of current immunotherapies and to define new treatment options [56•,57•].

Breast

Breast cancer (BC) is the most commonly diagnosed cancer in women and has the second highest incidence overall worldwide [58]. Cancers arising in the breast tissue are diverse in their (epi)genetic and morphological features as well as metabolic profiles. BC can be classified into three distinct molecular groups based on expression of hormonal receptors (estrogen and/or progesterone receptor positive), human epidermal growth factor receptor HER2 positive, and triple negative tumors (TNBC) - lacking estrogen receptor, progesterone receptor and HER2 [59]. This classification is the major characteristic that determines the therapeutic approach to treat the disease. However, it has been shown that even within each of those groups there can be considerable molecular heterogeneity.

The correlation between the abundance of CTLs and favorable disease outcome has recently been demonstrated in a cohort of 187 BC patients [60]. Furthermore, higher $CD8^+$ T cells infiltration can be used as a predictive factor of response to non-adjuvant chemotherapy for patients with HER^+ and TNBC. In regard to Treg cell infiltration, there is a considerable disparity among the different BC subtypes. Most studies find that high Treg cell infiltration correlates with poor disease outcome [61]. However, there are some studies which have found the opposite to be true in TNBC [62].

Breast cancer cell metabolism

Different types of BC also differ in their metabolic profiles (Figure 2). TNBC subtype primarily utilizes the Warburg effect metabolism characterized by high glucose uptake and lactate secretion even in the presence of oxygen [63]. Additionally, TNBC relies heavily on uptake and storage of exogenous fatty acids while ER^+ BC subtype prefers fatty acid synthesis (FAS) and oxidation. Furthermore, ER^+ BC subtype displays the reverse Warburg metabolic effect – where neighboring stromal cells are induced to undergo aerobic glycolysis and then transfer the catabolites to the cancer cells for them to use in OXPHOS to support their proliferation. $HER2^+$ cancer subtype can be characterized by a unique Warburg like metabolic effect which is heavily reliant on FAS and has been shown to have increased levels of stored fatty acids [64]. Considering that lipid metabolism differs substantially between different subtypes Eiriksson *et al.* sought to determine if the differences in the lipidome could be used as a marker to distinguish the different subtypes for diagnostic purposes [65•]. Analysis of the lipidome of different BC subtype cell lines showed that cells of the luminal BC subtype (ER and PgR positive) have increased abundance of triacylglycerols (TG) with moderate or multiple unsaturated fatty acid chains, while these lipids are significantly downregulated in HER2 and TNBC subtypes [65•]. Furthermore, concerning lipid metabolism, cholesterol biosynthesis was shown to be essential for breast cancer stem cell propagation [66•]. Additionally, inhibition of cholesterol synthesis enzymes leads to significant reduction in TNBC mammosphere growth and propagation [66•], thus providing evidence for the potential of targeting cholesterol synthesis pathway in the treatment of TNBC patients. Lipid metabolism reprograming has also been extensively linked to the development of resistance to kinase inhibitors in BC [67].

A recent study has shown that EMT in BC is associated with the attenuation of succinate dehydrogenase (SDH) [68•]. This enzyme is part of the mitochondrial electron transport chain and therefore an important player in energy metabolism. With this study Røsland *et al.* provide further proof of the importance of mitochondrial processes in EMT and highlight its potential as a therapeutic target. Additionally, inhibition of estrogen-related receptor α (ERRα) in TNBC cell lines leads to reduced pyruvate entry into the mitochondria resulting in cells becoming more dependent on glutamine and glucose oxidation and ultimately leads to cancer cells being more sensitive to GLS and PPP inhibitors [69]. This highlights the need to evaluate the interplay between the different metabolic factors and the potential of a cascade effect between different metabolic pathways when considering therapeutic approaches targeting cancer metabolism.

Since the development of metastasis correlates with poor prognosis, it is important to identify factors that contribute to a more invasive BC phenotype. Aldo-keto reductase AKR1B10 has been identified as one such factor. AKR1B10 promotes metastasis by limiting the oxidative

stress and enabling fatty acid oxidation (FAO) in the metastatic environment [70••]. This metabolic alteration has been shown to be important only for metastatic colonization but not for primary tumor growth, therefore AKR1B10 inhibition represents a potential treatment option for advanced BC. Additionally, high expression of AKR1B10 could be used to identify patients with a higher risk of metastasis. Even though the connection between metastasis and AKR1B10 expression has so far only been shown in BC, AKR1B10 is highly expressed in other cancer types, including lung [71] and pancreas [72], suggesting the possibility to explore this factor as a therapeutic target in multiple cancer types.

Considering the emerging new research, fatty acid metabolism stands out as the group of metabolic processes which have been shown to be most important for BC cell growth and development of resistance to many existing therapeutic approaches. Further research is necessary to determine how targeting different metabolic pathways can diminish cancer cell proliferation while at the same time boosting T cell fitness within the TME, ultimately leading to improved disease prognosis.

T cell metabolism in breast cancer

Out of the different BC subtypes, TNBC is considered as the one to most likely respond to ICI treatment. This is largely due to the fact that TNBC generally has a higher TIL infiltration [73], as well as high expression of PD-L1 [74], which is a direct target for this class of immunotherapy. In fact, based on the results of a recent clinical trial (NCT02425891) [75], the FDA granted accelerated approval for α-PD-L1 treatment using a monoclonal antibody in combination with chemotherapy for treatment of PD-L1 positive, unresectable, locally advanced or metastatic TNBC. More on the current state of immunotherapy in TNBC has been recently reviewed elsewhere [76].

STAT3 activation and $CD8^+$ T cell functions have been correlated in obesity-associated BC. Deletion of STAT3 in obese mice, who are known to spontaneously develop tumors, was shown to decrease FAO, increase glycolysis and improve $CD8^+$ T cell effector functions, leading to reduced BC incidence in these mice [77•]. Additionally, leptin, which is abundant in mammary adipocytes and surrounding fat tissue, was shown to inhibit $CD8^+$ T cell effector functions by activating STAT3, consequently inducing FAO and reducing glycolysis. Furthermore, leptin is one of the key adipokines produced by breast tissue adipocytes (Figure 1) that can promote BC stem cell self-renewal and chemo-resistance [78]. Therefore, targeting the leptin-STAT3-FAO pathway may result in enhanced anti tumoral $CD8^+$ T cell response in addition to inhibiting cancer cells in obesity associated BC (Figure 3).

Lipid chaperone fatty acid binding protein 5 (FABP5) expression is increased in tumor infiltrating Treg cells isolated from human BC [79••] and murine subcutaneous E.G7 tumor model [80••]. Curiously, *in vitro* studies show that inhibition of FABP5 leads to mitochondrial alterations and increased suppressive function of Treg [80••]. Because there is a limited amount of lipids in the tumor, it is believed that Treg cells increase FABP5 expression as an adaptive response to the low-lipid environment. This and other indications suggest that the metabolic needs of Treg cells, both *in vivo* and *in vitro*, are likely dynamic and depend on their activation stage as well as the nutrient microenvironment at the site of activation [80••].

A bioinformatics study, which analyzed gene expression data from 4921 cancer patients, determined that elevated expression of the hepatic lipase LIPC correlates with higher infiltration of both myeloid and lymphoid cells in several cancer types including breast, melanoma and NSCLC [81•]. In contrast high expression of aldehyde dehydrogenase seven family, member A1 (ALDH7A1) correlates with lower infiltration of immune effector cells. The mechanism governing these correlations is still to be investigated.

Autophagy is a cellular process linked to immune response in various infections and recently also to antitumoral $CD8^+$ T cell immunity. Mice with a deficiency in the genes essential for autophagy Atg5, Atg14, or Atg16L1, have a significant decrease in the growth of breast, prostate and colorectal tumors [82••]. Additionally, T cells with a deletion in the Atg5 gene exhibit a shift to a memory phenotype along with an increase of proinflammatory cytokines IFNγ and TNFα. *In vitro* and *in vivo* mechanistic studies done in syngeneic e0771 model of BC indicate that the suppression of autophagy caused by deletion of Atg5 leads to increased glycolysis in $CD8^+$ T cells and a decrease in the concentrations of S-adenosylmethionine (SAM) [82••]. This metabolic alteration consequently leads to changes in histone methylation, which is associated with increased expression of effector and metabolic genes, resulting in a memory T cell phenotype. Disruption of autophagy in T cells has therefore been proposed as a therapeutic strategy that could enhance immunotherapeutic effort in human cancers.

With the breakthrough of CRISPR/Cas9 technology, genomic screens represent a promising approach for new target discovery and the advancement of immunotherapy. One such screen was done on $CD8^+$ T cells selecting for tumor infiltration in mouse models of TNBC in the context of immunotherapy [83•]. This screen identified several genes associated with higher $CD8^+$ T cell infiltration, activation and effector functions. One of these genes is Dhx37, encoding for an RNA helicase, which, when deleted in $CD8^+$ T cells, improved ACT efficacy in an orthotopic BC mouse model utilizing the

syngeneic mouse TNBC cell line E0771. The use of more focused screening libraries, such as those targeting metabolic genes, could potentially lead to the discovery of even more promising druggable targets affecting T cell infiltration and fitness within the TME.

Prostate

Prostate cancer is the second most commonly diagnosed cancer type in men, behind LC, and represents a major cause of male morbidity and mortality. In 2018 alone, there were over 1.2 million new diagnosis reported worldwide and close to 360 000 deaths related to prostate cancer[58]. Despite some heterogeneity in prostate cancer among the patient population, there is a generalized phenotype that is common to all patients including some metabolic alterations observed in prostate cancer cells. These metabolic events are distinct between the different stages of disease - early versus late stage. The Gleason grading system is commonly used to diagnose and classify the severity of prostate cancer using histopathological examination and scoring of the cells biopsied based on its resemblance to healthy prostate cells. Score 6 is given to low-grade cancers, score 7 to intermediate, and score 8–10 indicates high-grade and most aggressive prostate cancer. Healthy prostate epithelium cells are known for the high accumulation of Zinc as well as citrate synthesis and a lack of citrate oxidation (Figure 1).

Depending on the characteristic of the tumor, prostate cancer patients are subjected to one or more treatments, including surgery, androgen deprivation therapy and chemotherapy. However, there are very limited treatment options for metastatic disease and immunotherapy has not been proven effective in prostate cancer. Prostate cancer is classified as a 'cold' tumor with minimal T cell infiltration, with several factors contributing to its non-inflamed phenotype. Prostate cancer is characterized by relatively few somatic mutations, compared to other cancer types, and consequently low expression of neoantigens that could trigger a T cell response. Other factors contributing to this non inflamed phenotype of prostate cancer are downregulation of MHC Class I expression, PTEN loss and a deficiency in IFN1 signaling [84]. It is possible that the use of drugs targeting metabolism could synergize and render these tumors susceptible to immunotherapy such as ICI. Despite this unfavorable TME, it has recently been shown that it is possible to isolate functional and anti-tumor reactive TILs from clinical prostate cancer samples [85•], therefore providing a rationale for further investigation into TIL immunotherapy for prostate cancer.

Prostate cancer cell metabolism

In cancer cells there is a metabolic shift in this respect and malignant prostate cells convert to a citrate oxidizing phenotype accompanied by a decrease in zinc concentrations (Figure 2). In line with this, prostate cancer cells have been shown to utilize the TCA cycle and OXPHOS more compared to benign prostate cells. Prostate cancer cell metabolism is unique in that it does not generally display the Warburg effect in glucose metabolism that most other cancers adhere to. The Warburg effect and increased glucose metabolism together with lactate secretion can only be observed in the late stages of disease and after accumulating multiple mutations [5]. Using *in vivo* hyperpolarized MRI imaging followed by tissue histopathology, Granlund *et al.* demonstrate that tumors with increased Gleason grades had significantly increased levels of lactate [86]. Furthermore, increased metabolism of pyruvate to lactate is augmented in tumors with homozygous deletion of the PTEN gene.

The remodeling of OXPHOS in prostate cancer has recently been linked to mitochondrial DNA (mtDNA) mutations and differentially expressed mitochondrial genes, especially in high-grade cancers [87•]. Specifically, increased mutations in mitochondrial Complex I encoding genes, correlate with a significant increase in succinate oxidation.

Prostate epithelial cells exhibit a high rate of acetylated polyamine secretion in the prostate lumen. This behavior is maintained by prostate cancer cells, who additionally also require high concentrations of intracellular polyamines leading to an increased demand for connected metabolic pathways [88]. An interesting new therapeutic approach has been proposed to target this metabolic vulnerability of prostate cancer [89••]. In order to cause apoptosis, metabolic stress is induced in prostate cancer cells by using a polyamine analog N^1,N^{11}-bisethylnorspermine (BENSpm) which increases spermidine/spermine N1-acetyltransferase (SSAT) activity resulting in a higher acetylated polyamine export. At the same time, cells are treated with a small molecule inhibitor (MTDIA) of methylthioadenosine phosphorylase (MTAP), the key enzyme in the methionine salvage pathway (MSP), which is necessary for the cell to alleviate this stress, ultimately leading to cell death. This approach was shown to be effective in different prostate cancer cell models *in vivo* and *in vitro* [89••].

Androgen receptor signaling leads to increased proliferation and survival of prostate cancer cells. This is why androgen deprivation therapy (ADT) is one of the main treatment options for metastatic prostate cancer or upon relapse to help control the spread of the disease. Androgen receptor has also been shown to play a central role in metabolic reprograming of prostate cancer cells. Androgen receptor signaling upregulates the expression of Dynamin related protein 1 (DRP1) in the mitochondria, leading to increased pyruvate transport into the mitochondria and resulting in augmented OXPHOS and lipogenesis [90]. Moreover, high expression of DRP1 in prostate cancer cells correlates with poor disease

prognosis [90]. Another molecular player involved in the promotion of prostate cancer progression in connection to androgen receptor (AR) signaling is Sirtuin7 (SIRT7) [91•]. SIRT7 depletion significantly reduces prostate cancer cell proliferation and androgen-induced autophagy *in vitro* and *in vivo*. Furthermore, upregulation of SIRT7 correlates with the expression of AR as well as prostate-specific antigen (PSA) and could therefore be used as a prognostic marker for prostate cancer in addition to being a potential drug target.

T cell metabolism in prostate cancer

Hypoxia in prostate tumors has been associated with poor prognosis [92]. T cells are largely excluded from hypoxic zones within the tumor therefore drugs reversing hypoxia represent a promising treatment strategy to increase T cell infiltration and improve their fitness in the prostate TME. In fact, hypoxia-activated prodrug TH-302 was shown to eliminate hypoxia in the transgenic adenocarcinoma of the mouse prostate (TRAMP) derived TRAMP-C2 mouse model [93•]. Additionally, treatment of TRAMP-C2 mice with a combination of both TH-302 and immune checkpoint blockade (α-CTLA-4 and α-PD-1) acts synergistically to cure over 80% of the tumors.

Indoleamine 2,3-dioxygenase (IDO) is the rate limiting enzyme of the metabolic reaction in the metabolism of tryptophan, which is known to be essential for T cell activation. Excessive expression of IDO in cancer cells leads to the depletion of tryptophan and the accumulation of associated metabolites, which are known to inhibit T cell expansion and activation as well as aid in the recruitment of Treg cells and other suppressive cell types [94]. IDO expression was shown to correlate with higher stages of prostate cancer progression and was indicative of resistance to immunotherapy [95]. Furthermore, IDO expression is higher after anti-tumoral vaccine or α-PD-1 treatment. Taken together these results suggest that IDO expression could be a mechanism of resistance to immunotherapy, therefore future research including IDO inhibitors in combination with immunotherapy should be explored. Considering that IDO inhibition has previously been studied as a potential addition to immunotherapy in different cancer types without much success [96], it is perhaps worth exploring therapeutic effect of IDO inhibition in combination with different types of therapies, potentially other metabolic targets in addition to immunotherapy.

Several clinical trials are exploring the possibility of immunotherapy for the treatment of prostate cancer. The first cancer vaccine for the treatment of metastatic castrate resistant prostate cancer was approved by the FDA in 2010 and has been shown to prolong overall survival by an average of 4 months [97]. Numerous other cancer vaccines and different immunotherapeutic approaches have been tested but ultimately none have led to a dramatic improvement in prognosis for patients with advanced prostate cancer [98]. Therefore, research into new and improved therapeutic options are urgently needed. Metabolic targets in both cancer and immune cells represent a promising avenue to explore.

Conclusion

The idea of targeting metabolism in cancer therapy has been around for a long time. In fact, many standard chemotherapy drugs have metabolic targets. However, systemic administration of many of these metabolic drugs come with significant off target toxicity since they target pathways that all healthy and cancer cells require for proliferation (such as nucleotide synthesis). Over the past decade immunotherapy has established itself as a promising treatment option for cancer patients. However, it has become clear that on its own, immunotherapy in the form of ICI and ACT is not successful for most patients. The major limitation of these approaches is the fact that T cell (and other anti-tumor immune cell) fitness is generally compromised within the harsh TME. Moreover, the ability of immunotherapy to unleash the immune system against the tumor comes with the draw back to elicit systemic immune reactions. For example, patients treated with ICI may experience colitis, hepatitis, myocarditis, pneumonitis or neurotoxic effects all due to the excessively activated immune system [99]. In an estimated 0.3–1.3% of cases these complications can be fatal. Cancer cells deplete crucial nutrients from the TME and secrete factors that actively inhibit effector T cell function. As discussed in this review, the metabolic features of the TME can vary significantly between the different tissues of origin and even between cancer subtypes within the same tissue. Consequently, T cell response in different cancers differs as well due to the diverse adaptation of T cells and their fitness within the specific TME. Targeting specific metabolic factors, in cancer cells and/or T cells, based on the cancer tissue of origin and histotype represents a promising treatment option to complement immunotherapy. There are several approaches to consider in this respect. Metabolic drugs could be used in combination with immunotherapy as an *ex vivo* preconditioning approach in order to induce T cell metabolic adaptation to the specific TME and increase their infiltration and fitness which in turn would lead to improved therapeutic efficiency of ACT. By treating TILs or engineered T cells *ex vivo* before ACT, off target toxicity related to metabolic reprograming could be avoided. Alternatively, drugs targeting metabolism could be used as part of the systemic immunotherapeutic regimen, where patients would only need to be treated for a short period of time, allowing the rewiring of the TME, therefore creating an opportunity for T cells, re-invigorated through an immunotherapeutic approach, to enter the TME and act against cancer cells. This would allow for a more tissue specific response, possibly preventing adverse reactions related to the systemic 're-activation'

of the immune system. Mirroring this line of thinking, if in cancer targeting metabolism holds the potential to increase T cell fitness and to inhibit Treg cell suppressive capacities, in case of autoimmune disorders dampening the metabolic demands of reactive CD8+ T cells or reinforcing the fitness of Treg cells can disclose new strategies in the cure of this type of diseases.

Conflict of interest statement

Nothing declared.

CRediT authorship contribution statement

Nina C Flerin: Conceptualization, Writing - original draft, Writing - review & editing. **Federica Cappellesso:** Writing - original draft, Writing - review & editing. **Samantha Pretto:** Writing - original draft, Writing - review & editing. **Massimiliano Mazzone:** Conceptualization, Writing - review & editing, Funding acquisition, Supervision.

Acknowledgements

M.M. was supported by an ERC Consolidator grant for the project ImmunoFit (773208), Research Foundation Flanders (Fonds Wetenschappelijk Onderzoek - FWO) projects G0D1717N and G066515N, Belgian Association against Cancer (Stichting tegen Kanker) project 2014-197. FC has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 766214. SP is supported by FWO doctoral grant 1S68420N. We would like to acknowledge Servier Medical Art for image elements used in the figures (licensed under a Creative Common Attribution 3.0 Generic License).

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