The insulin and insulin-like growth factor receptor family in neoplasia: an update

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Abstract | Although several early phase clinical trials raised enthusiasm for the use of insulin-like growth factor I receptor (IGF1R)-specific antibodies for cancer treatment, initial Phase III results in unselected patients have been disappointing. Further clinical studies may benefit from the use of predictive biomarkers to identify probable responders, the use of rational combination therapies and the consideration of alternative targeting strategies, such as ligand-specific antibodies and receptor-specific tyrosine kinase inhibitors. Targeting insulin and IGF signalling also needs to be considered in the broader context of the pathophysiology that relates obesity and diabetes to neoplasia, and the effects of anti-diabetic drugs, including metformin, on cancer risk and prognosis. The insulin and IGFI receptor family is also relevant to the development of PI3K–AKT pathway inhibitors.

C-peptide
Insulin is produced as preproinsulin that consists of an A-chain, a C-peptide, a B-chain and a signal sequence. The signal sequence is cleaved to produce proinsulin, and the C-peptide is cleaved leaving the A-chain and B-chain to form insulin.

As detailed in two previous Reviews in *Nature Rev. Cancer*[1,2], justification for developing therapies that target the insulin and insulin-like growth factor I (IGFI) receptor family (IIRF) included initial evidence that the IGFI receptor (IGF1R) is necessary for the transforming ability of several oncogenes[3]; that insulin or IGFI can stimulate the proliferation of tumour cells *in vitro*[4]; and that genetic manipulations that reduced IGF signalling can lead to decreased tumour growth in mouse models[1]. In addition, epidemiological evidence indicates that insulin secretion rate (reflected by c-peptide levels) and IGFI levels influenced cancer risk and/or cancer prognosis (reviewed in REFS 1,5). There is also a plausible hypothesis that the substantial adverse effect of obesity on cancer burden might be mediated in large part by insulin. This also contributed to the rationale to study therapies with the potential to reduce insulin signalling, particularly in subjects with increased insulin levels. Of the dozens of drug candidates synthesized, those that demonstrated significant activity in preclinical models were taken forwards for clinical evaluation. However, the outcomes of Phase III clinical trials have been somewhat disappointing. Possible reasons for this, potential next steps and relevance to other areas of drug development in oncology are the subject of this Review.

Receptors and signalling

Insulin-like signalling is ancient[6]. Although the medical importance of insulin in diabetes led to its discovery, in evolutionary terms the regulation of carbohydrate metabolism is a fairly recent and specialized function of insulin. Primitive organisms use insulin-like signalling systems to control cell proliferation and survival. These functions remain important in higher organisms, and have particular relevance to oncology. The molecular evolution of IGFI, IGFII and insulin as individual ligands, and their receptors, has been reviewed[7,8].

The insulin receptor and the IGF1R are members of the tyrosine kinase class of membrane receptors, and are homologous to oncogenes of the tyrosine kinase class[9]. The insulin receptor exists in two splice variant isoforms; the 'B' isoform recognizes only insulin, but the 'A' isoform, which is the isoform that is most commonly expressed by tumours, recognizes both insulin and IGFII[10]. The IGF1R and the insulin receptor are complex molecules. Each gene product is processed extensively and finally forms glycosylated α-chains and β-chains that associate to form a 'half' receptor; two half receptors then associate to form a holoreceptor. Interestingly, heterodimers comprised of a half insulin receptor and a half IGF1R can form, and these are known as hybrid receptors[10,11]. As most cancers express both the insulin receptor and the IGF1R genes, they display many of the receptor species shown in FIG. 1, rather a single receptor type. At the cellular level, signalling downstream of insulin receptors and hybrid receptors is similar but not identical. In each case, the kinase activity of the receptor leads to phosphorylation of members of the insulin receptor substrate (IRS) family of proteins,

At a glance

- Preclinical evidence for a role of insulin and insulin-like growth factor (IGF) signalling in promoting neoplastic growth is impressive.
- Several different targeting strategies for the insulin and IGFI receptor family exist, and dozens of drug candidates have shown activity in model systems.
- Phase III clinical trials have so far been undertaken only with IGFI receptor-specific antibodies. Although the final results have not yet been published, disappointing reports have been presented for some of these trials. Future trials may differ by incorporating predictive biomarkers, by using rational combination therapy approaches and by using other pharmacological approaches to targeting, such as anti-ligand antibodies or tyrosine kinase inhibitors.
- The insulin and IGFI receptor family may be involved in resistance mechanisms to therapies that target other signalling nodes in cancer cells, suggesting that there may be situations in which co-targeting will confer benefit.
- The insulin and IGFI receptor family is now known to have a role in the important relationships between macronutrient intake and cancer, diabetes and cancer, and obesity and cancer.
- Biguanides, such as metformin, which is widely used in diabetes treatment, have been reported in hypothesis-generating retrospective population studies of subjects with diabetes to be associated with reduced cancer burden. These agents lower insulin levels if they are increased, and have a variety of effects on cellular signalling and cellular metabolism. However, there are gaps in knowledge related to their pharmacokinetics and mechanisms of action that require elucidation.

and this leads to activation of PI3K, AKT and various downstream networks[12]. However, different cell types use this control system to regulate different processes. For example, a major consequence of pathway activation in the liver is the inhibition of gluconeogenesis and the activation of glycogen storage. By contrast, epithelial cells do not express gluconeogenic enzymes and consequences of pathway activation include the stimulation of proliferation and the inhibition of apoptosis.

Ligands and receptor activation

It is important to recognize that IIRF members are widely expressed on neoplastic and normal tissues[13–16]. Although receptor levels in cancers are sometimes higher than the levels seen in normal tissues, gene amplification associated with large increases in receptor number (such as that seen in the case of ERBB2 (also known as HER2 and neu) is rare. Furthermore, activating mutations and ligand-independent activation of these receptors are the exception rather than the rule. Thus, ligand-mediated receptor activation is necessary for the IIRF to influence carcinogenesis or cancer behaviour. Abnormal autocrine or paracrine expression of ligands, particularly IGFII, is common in many malignancies[17], and the presence of such loops may denote 'addiction' to IIRF activation. Of course, another source of ligand is systemic, and there is evidence that variations in circulating ligand levels influence the degree of activation not only of IIRF members in classic insulin- or IGF-sensitive tissues, but also of these receptors in neoplastic tissues[18,19].

Insulin expression is confined to specialized pancreatic β-cells, and under normal circumstances it is tightly regulated by the level of circulating glucose. In contrast to epidermal growth factor and other tissue growth factors that are relevant to neoplastic disease, insulin functions as a classic hormone, influencing tissues remote from its site of production. Abnormal autocrine production of insulin by cancers is uncommon. Insulin-stimulated glucose uptake by classic insulin-sensitive organs (liver, muscle and adipose tissue) reduces circulating glucose levels. Although many cancers display high rates of insulin-independent glucose uptake, there is evidence that, in some cases, tumour glucose uptake is also insulin-stimulated[18–20]. In early type 2 diabetes, insulin resistance of classic insulin-target organs (often induced by excess calorific intake and attributable to overactive cellular feedback pathways that regulate insulin action; for example, by serine phosphorylation of IRS proteins[21]) leads to hyperinsulinaemia. Initially, these increased levels of insulin are sufficient to overcome insulin resistance and to avoid hyperglycaemia. However, hyperglycaemia eventually occurs not only because of increasing insulin resistance but also because of decreasing insulin output by pancreatic β-cells. The degree to which cancers in patients with type 2 diabetes share the insulin resistance of their host remains to be determined, but any transformed cells that remain more insulin sensitive than liver, muscle or adipose when these tissues become insulin resistant would be predicted to be growth-stimulated by the hyperinsulinaemia present. There is some experimental support for this possibility[22,23] (FIG. 2). There is also evidence that variations between individuals in rates of insulin secretion (as reflected by c-peptide levels) influence cancer risk[24] and prognosis[25].

In contrast to insulin, IGFI and IGFII are widely expressed by many cell types, and autocrine expression by transformed cells is common. IGFs have characteristics of both hormones and tissue growth factors, as responsive cells may respond to locally produced ligands and/or to ligands delivered via the circulation. The main site for production of IGFs is the liver. Although many factors influence hepatic production, growth hormone is the dominant stimulatory influence, particularly for IGFI. There is substantial variation between normal individuals in circulating levels of IGFI, and twin studies demonstrated that this variation is approximately 50% genetically determined, with the balance of variability attributable to lifestyle factors[26]. Subsequent studies provided evidence that dozens of genes each contribute to the genetic portion of the variability. Importantly, many population studies (reviewed in REF. 1) and experimental studies (for example, REF. 27) indicate that interpersonal variability in IGFI levels influences cancer risk. An additional level of control of IGF biological activity is provided by the family of high-affinity IGF-binding proteins (IGFBPs)[28]. The complexity of insulin and IGF physiology (described above) has to be recognized not only in the design of clinical trials of agents that directly target insulin or IGFI signalling, but also in the use of agents, such as metformin and PI3K pathway inhibitors, that perturb insulin and IGFI actions both in the cellular context and at the level of whole-organism physiology (FIG. 2).

Clinical trials: disappointments and clues

Targeting strategies. Anti-receptor antibodies, anti-ligand antibodies and small-molecule receptor kinase inhibitors have all been used to target the IIRF, and certain drug candidates from each of these classes were considered promising enough in preclinical models to

Type 2 diabetes
Diabetes that initially arises as a result of insulin resistance in tissues such as the liver, muscle and fat, rather than through primary loss of β-islet cells in the pancreas.

Hyperinsulinaemia
High concentrations of insulin circulating in the blood.

Hyperglycaemia
High concentrations of glucose circulating in the blood.

be taken forwards to clinical trials[29,30]. The anti-receptor antibodies have been the subject of the most intense clinical research activity, extending to Phase III trials, while the other classes are currently in Phase I or Phase II trials. Additional agents under study include picropodophyllin (AXL1717), which seems to inhibit IGF1R signalling by an incompletely characterized mechanism[31].

The various anti-receptor antibodies that have been developed have many features in common, but they are not identical in terms of antibody subtype, half-life and so on. All these agents were designed to spare the insulin receptors (as insulin receptor blockade was considered to be too dangerous), and this has been accomplished. They all interfere with ligand binding to the IGF1R and also interfere with ligand binding to hybrid receptors, although this is less well documented. Despite the lack of interference with insulin binding, the use of these antibodies is associated with hyperglycaemia and hyperinsulinaemia, which can be severe, particularly if patients are also receiving steroids[30]. As we predicted[1], the use of these agents leads to a major increase in growth hormone secretion, as the pituitary attempts to compensate for the perceived lack of IGF biological activity. This leads not only to increases in circulating IGFI levels (which can reach levels tenfold above normal) but also to growth hormone-induced insulin resistance, which accounts for the observed hyperglycaemia and hyperinsulinaemia (FIG. 2) in treated patients. Another potential mechanism by which IGF1R blockade can lead to hyperglycaemia is related to the role of IGFI in pancreatic β-cell physiology[32].

Although the initial development of small-molecule tyrosine kinase inhibitors involved attempts to achieve IGF1R specificity[33], these agents tend to inhibit all members of the IIRF *in vivo*. Early clinical experience suggests that these agents are safer than was originally anticipated, and their broader range of receptor inhibition may be a therapeutic advantage. Why do such agents not cause severe metabolic toxicity that is similar to uncontrolled diabetes? One possibility is that, at the dosages used, insulin receptor signalling is incompletely inhibited. However, there is preliminary evidence that more complex pharmacokinetic issues may be involved. It seems that the drug concentrations that are achieved are fairly low in muscle, which is a major site of insulin-stimulated glucose disposition. Therefore, in this tissue, insulin receptor function is fairly intact, perhaps accounting for a modest rather than a severe effect of these kinase inhibitors on systemic glucose metabolism[34]. Nevertheless, insulin levels are increased in patients treated with IIRF kinase inhibitors, implying that this compensation is necessary to achieve sufficient insulin activity to control blood glucose. An important question concerns the possibility that compensatory hyperinsulinaemia may limit efficacy. This is a complex issue that must take into account the varying levels of the kinase inhibitor in neoplastic tissue and various normal insulin-target tissues.

Anti-ligand antibodies have a high affinity against both IGFI and IGFII, but they do not cross-react with insulin. At least one of these antibodies[35] is in clinical trials. An interesting question concerning these agents relates to the IGFBPs, which normally bind greater than 90% of circulating IGFs. In the presence of ligand-specific antibodies, one may speculate that ligand–antibody complexes would replace ligand-binding protein complexes as the dominant circulating ligand species, resulting in high levels of free IGFBPs. There is evidence that free IGFBPs have antiproliferative activity that is independent of their IGF-binding capacity[28]; so, this approach may have physiological effects that are distinct from those of the receptor-specific antibodies or the IIRF kinase inhibitors. There is evidence that IGFII can act as a ligand for the A isoform of the insulin receptor, and that this can lead to an autocrine stimulation loop that would not be interrupted by IGFIR-specific antibodies, at least for those cells that display a sufficient number of holoinsulin receptors of this isoform[36] (FIG. 1). Such autocrine loops could be interrupted by ligand-specific antibodies, provided sufficient tissue concentrations were achieved.

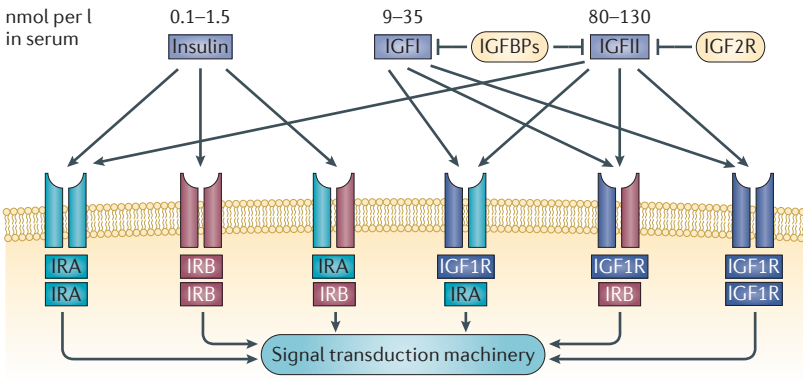

Figure 1 | **The insulin and IGFI receptor family.** The insulin receptor exists in two splice variant isoforms, the 'A' isoform (IRA) and the 'B' isoform (IRB), but the insulin-like growth factor I (IGFI) receptor only has one. 'Half' receptors dimerize to form six receptor species, and the receptors vary in their ligand affinity. Classic insulin-target organs preferentially express the insulin receptor B isoform, which is only stimulated by insulin, and classic IGFI receptor-responsive tissues preferentially express the IGFI receptor (IGF1R). Cancers and non-classic target tissues may express both the insulin and the IGF1R genes, and may display many receptor species. IGFI and IGFII can be expressed in endocrine, paracrine or autocrine manners. The liver is their main site of production, and abnormal auocrine loops are common in cancer. By contrast, insulin production is confined to pancreatic β-cells. Insulin circulates at much lower concentrations than the IGFs, but insulin has direct access to its target tissues, in contrast to the IGFs, which may be diverted from their receptors by IGF-binding proteins (IGFBPs), or, in the case of IGFII, by the IGF2R, which targets the ligand for degradation without signal transduction.

Results to date. More than 100 clinical trials examining the hypothesis that targeting the IIRF will be useful in cancer treatment have been undertaken, and many are ongoing. It is beyond the scope of this Review to assess them individually. Reviews of the status of the trials (such as REFS 29,30) quickly become outdated, and meeting abstracts or online resources, such as the ClinicalTrials.gov website (see Further information), provide more current information.

Although some Phase II studies of IGFIR-specific antibodies showed activity with little toxicity, and there were reports of major responses[37–39], initial Phase III trial reports have documented a lack of efficacy, together with

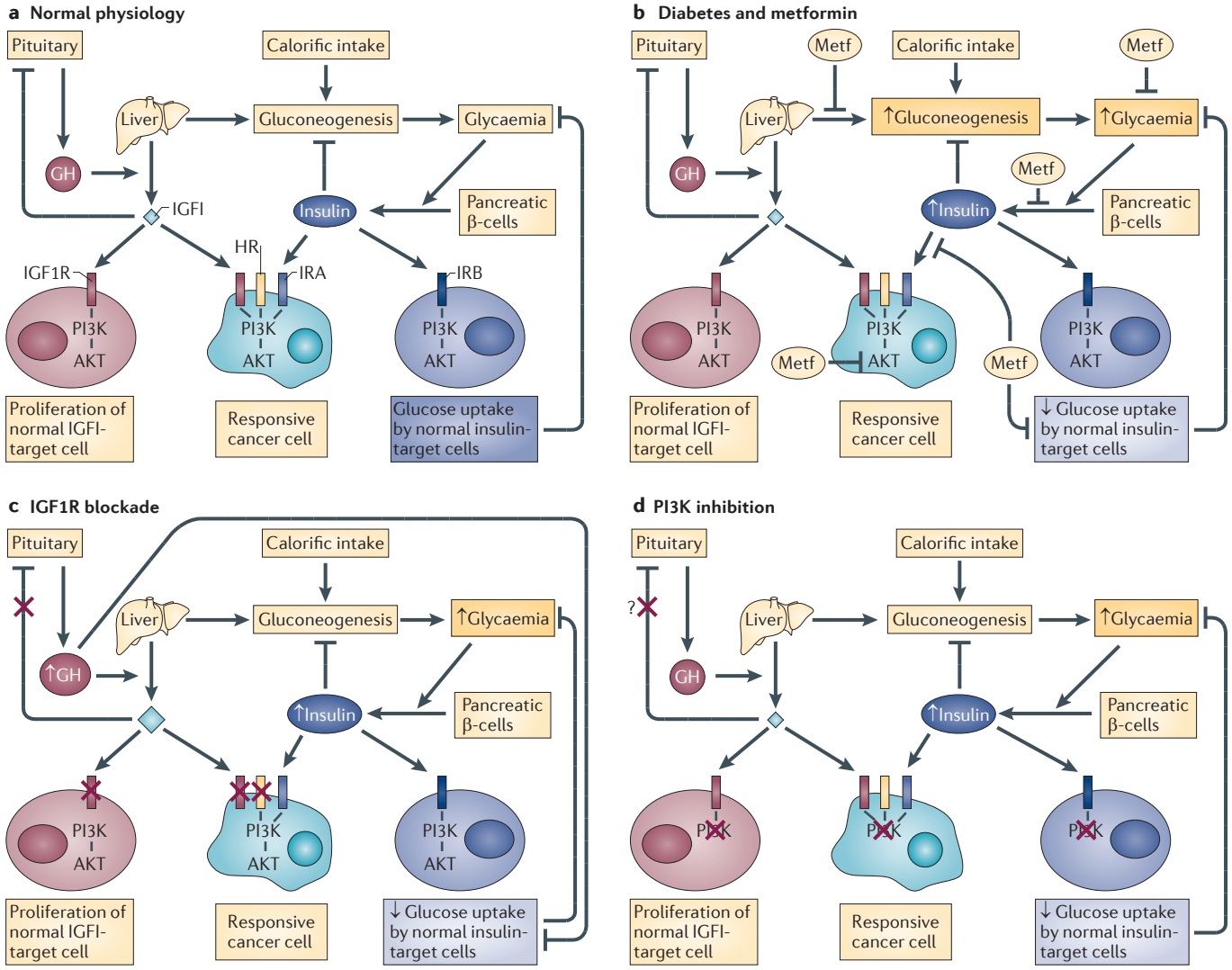

Figure 2 | **Perturbations of insulin and IGFI physiology by type 2 diabetes, metformin, IGF1R antibodies and PI3K inhibitors. a** | A simplified overview of some of the more important regulatory networks of insulin and insulin-like growth factor (IGF) physiology is shown, although many aspects (such as the role of glucagon) are excluded. **b** | One of the fundamental aspects of type 2 diabetes is the reduced uptake of glucose by normal insulin-target cells, which leads to hyperglycaemia. This in turn leads to hyperinsulinaemia, as the pancreatic β-cells attempt to lower blood sugar levels. Increased glucose release from the liver also contributes to the hyperglycaemia of type 2 diabetes. Metformin (Metf) acts in the liver to inhibit gluconeogenesis. This reduces hyperglycaemia, and lowers the abnormally high insulin levels that are characteristic of type 2 diabetes. This reduces insulin stimulation of the subset of tumours that are insulin-responsive. If metformin is present at high enough concentrations, tumours that can actively take up metformin through the expression of the organic cation transporter 1 might also be inhibited by mechanisms that are related to the direct actions of metformin. **c** | The effect of blockade of IGFI receptor (IGF1R) is shown. Receptor signalling is inhibited on both normal and neoplastic IGF1R-positive cells, and also on IGF1R-positive cells in the hypothalamic–pituitary axis that are involved in the feedback inhibition of IGFI on growth hormone (GH) secretion. This results in substantial increases in GH, which stimulates the liver to increase IGFI production and also causes insulin resistance in insulin-target tissues, which raises glucose levels, and thereby leads to increases in insulin production. Note that the IGFI receptor family tyrosine kinase inhibitors have similar effects, but they also block insulin receptors. **d** | The effects of PI3K blockade are shown. Most PI3K inhibitors inhibit signalling in all tissues, although this is influenced by both pharmacokinetic factors and specificity for PI3K subtypes. Although this can have tumour growth-inhibitory effects, it also can reduce glucose uptake by insulin-target organs, resulting in hyperglycaemia, which leads to increased insulin levels. It is unclear the extent to which hyperinsulinaemia may attenuate the consequences of PI3K blockade, but in cases in which PI3K inhibition results in normal blood sugar levels with hyperinsulinaemia, it is possible that, at least in certain tissues, the hyperinsulinaemia does reduce the effectiveness of the blockade. There is experimental evidence that blockade of signalling downstream of the insulin receptor can lead to increased receptor expression, which may attenuate the effect of the blockade. The extent to which PI3K inhibitors may act to limit IGF1R-mediated feedback inhibition of growth hormone secretion, leading to increases in growth hormone and IGFI, has not yet been well defined. IRA, insulin receptor A isoform; IRB, insulin receptor B isoform; HR, hybrid receptor.

metabolic toxicity, chiefly hyperglycaemia[40]. Full results of completed Phase III studies have not yet been published, but there has already been considerable discussion about the interpretation of the currently available results. One view is that the negative Phase III data are sufficient to justify the abandonment of further investigation of therapeutic targeting of the insulin and IGFI receptor family for all indications, regardless of the targeting strategy. At the opposite extreme, some would limit the conclusion to the specific demonstration that particular IGF1R-specific antibodies, such as figitumumab[40], have no efficacy in unselected patients for the specific indication examined (for example, enhancement of benefit of chemotherapy for non-small-cell lung cancer), but allow for the possibility that this agent might be useful for other indications, possibly in other combinations, especially when patients were selected by the use of predictive biomarkers. Some would argue that negative Phase III results with certain IGF1R antibodies have no implications for other antibodies that are directed against this receptor, and certainly are not relevant to alternative targeting strategies, such as ligand-specific antibodies or receptor kinase inhibitors. This wide range of opinions is further illustrated by the fact that some pharmaceutical companies are closing insulin and IGF receptor drug development programmes but others are initiating or continuing trials. There are three areas (discussed below) that require urgent investigation if targeting the insulin and IGF1R family is to be taken forwards.

Predictive biomarkers. There are clear precedents in which the use of a predictive biomarker has been essential to define a subset of patients for whom a particular therapy is applicable, ranging from the classic case of trastuzumab for ERBB2-positive breast cancer to the more recent example of crizotinib for the small subset of lung cancers that are driven by ALK-fusion proteins[41]. None of the clinical trials of agents that target the IIRF has made use of predictive biomarkers because none had been defined when these trials were initiated. Recently, using data from the initial trials, candidate predictive biomarkers have been identified[42–44]. Some of them are supported by preliminary clinical evidence, but none has been validated.

Among these candidate predictive biomarkers is the pretreatment level of circulating IGFI[42–44]. Data to support this come from the evaluation of Phase II, rather than Phase III, studies, so no definitive conclusions are available, but it is intriguing that there is evidence that confining treatment to subjects with higher free IGFI levels would reduce toxicity and increase efficacy. The rationale offered for these findings is that tumours that arise in hosts with higher circulating ligand concentrations are more likely to become dependent on or even addicted to IGF1R activation, and are, therefore, more likely to respond to the interruption of signalling. Additional candidate predictive biomarkers include receptor levels and the presence of autocrine loops, or any deregulation in signal transduction machinery that would confer an exaggerated response to receptor activation. The presence of autocrine loops is fairly easy to assess in tumour specimens by measuring the expression of receptors and ligands. The use of this candidate predictive biomarker will require one to consider the efficacy of agents for tumours that are stimulated by circulating ligands from endocrine sources compared with those that are stimulated by locally produced ligands. Various drug candidates might differ in their efficacy depending on ligand source. For example, higher tissue levels of ligand-specific antibodies may be required to inhibit the growth of tumours that have strong autocrine production compared with those that rely on circulating ligands. Although it is likely that the presence of autocrine loops indicates a degree of dependency of tumours on the signalling pathway, it could be that autocrine loops are a marker of sensitivity for those agents capable of interrupting such loops, or that they are a marker of resistance for agents that are capable of attenuating receptor activation only in response to circulating ligands. Other candidate predictive biomarkers, such as the presence of certain transforming fusion proteins that seem to have a requirement for IGF1R activation[45], are under investigation. By contrast, although not yet investigated in clinical trial specimens, it is plausible that the presence of activating mutations downstream of the IGF1R, such as those resulting in constitutive activation of PI3K, would confer resistance to IIRF targeting.

Resistance mechanisms. There are probably many cancers that have evolved to such a degree that their behaviour is constitutively aggressive and uninfluenced by growth signals. For such tumours, targeting the insulin receptor and IGF1R (or any other receptor kinase) will be ineffective. Similarly, some cancers are driven by other receptors (such as ERBB2) to such an extent that insulin and IGF signalling become irrelevant, and these tumours would also be predicted to be resistant. There may be situations in which cancers are dependent on insulin or IGF1R activation, but, owing to processes such as insulin receptor-mediated resistance to IGF1R targeting[36,46,47], or the presence of a strong autocrine loop, particular targeting strategies might not be effective. Finally, it remains to be determined whether systemic endocrine compensatory responses to insulin or IGF targeting, such as increased levels of growth hormone, insulin or IGFI, can limit efficacy. There might also be situations in which the activation of the epidermal growth factor receptor (EGFR) represents a resistance mechanism limiting the efficacy of IGF1R targeting[48].

Combination therapies. Many targeted therapies are routinely used in combination with other agents, including, as a classic example, trastuzumab. Most trials with agents that target the IIRF have involved combinations, but in general these combinations have not been selected on the basis of a specific synergy demonstrated preclinically, but rather on a pragmatic approach involving the addition of a drug candidate to a current standard regimen that has some existing activity, but where there is a clear need to improve efficacy. Further preclinical studies could guide clinical trial design in this area and offer advantages over a strictly pragmatic approach.

Synthetic lethality experiments suggest co-targeting partners for agents that inhibit insulin and/or IGFI signalling[49]. There is evidence that resistance mechanisms to oestrogen deprivation treatment[50], approved targeted therapies[51–54], radiotherapy[55] or cytotoxic agents[56] involve insulin and IGF1R signalling. Combinations involving BRAF inhibitors[57,58] for melanoma deserve investigation. An additional example is provided by recent preclinical data indicating that insulin can stimulate local androgen production by prostate cancer cells[59], which suggests the possibility of using agents that target IIRF with castration and/or inhibitors of androgen synthesis. Similarly, co-targeting the IGF1R and the oestrogen receptor was found to be superior to using only single agents in a laboratory breast cancer model[60]. Data concerning the roles of the insulin and IGFI receptor family in mediating resistance to mTOR inhibitors[61] are also of considerable interest.

IIRF and inhibitors of PI3K, AKT and mTOR

PI3K, AKT and mTOR are the subject of major drug development efforts in oncology. These targets are downstream not only of the insulin receptor, but also of other receptor tyrosine kinases. Thus, successful targeting of these signalling nodes has the potential to not only eliminate the activation of an important part of the signalling networks downstream of the IIRF, but also to decrease key proliferative and survival signals that are initiated by other receptor tyrosine kinases, as well as signals that result from activating PI3K mutations. However, no drug candidates in these classes are administered in a manner that is expected to be tumour-specific; so, the inhibition of signalling downstream of IIRF members in normal tissues is to be expected. This inhibition could have many subtle effects on host physiology, but would be predicted to have an obvious effect on carbohydrate metabolism owing to drug-induced insulin resistance. A key concept is that, of the many receptor tyrosine kinase signalling systems that converge on PI3K, *in vivo* inhibition of this signalling node is likely to have the most substantial systemic effects as a consequence of interfering with insulin signalling, and thus with carbohydrate metabolism (FIG. 2). This is expected because, although many receptors upstream from PI3K function only as tissue growth factors, insulin and IGF signalling have major regulatory roles in whole-organism endocrinology, in addition to their roles as tissue growth factors. Thus, blockade of PI3K signalling is anticipated not only to cause compensatory changes at the cellular level, which may limit therapeutic efficacy[62], but also to lead to alterations in endocrine regulation at the whole-organism level. Indeed, drug-induced hyperglycaemia and/or hyperinsulinaemia can be regarded as useful and conveniently measured pharmacodynamic markers confirming that a PI3K-targeting drug is having an effect, although this would indicate inhibitory activity on classic insulin-target tissues rather than the intended inhibition of the same target in neoplastic tissue. PI3K or AKT inhibition would be expected to initially lead to hyperglycaemia and, secondarily, to compensatory hyperinsulinaemia. A finding of hyperinsulinaemia without hyperglycaemia would imply that the agent impaired signalling downstream of the insulin receptor, but that the compensatory hyperinsulinaemia was sufficient to restore insulin signalling to a degree that improved glucose uptake in classic insulin-responsive tissues. A finding of hyperinsulinaemia with hyperglycaemia implies a more complete inhibition of signalling. Experimental models are being used to determine the extent to which PI3K inhibition can be overcome by hyperinsulinaemia: important variables to be considered include the concentration of the inhibitor; the peak concentrations and time course of exposure to insulin or IGFI; the number of insulin receptors; and calorific intake.

If ongoing research demonstrates situations in which compensatory hyperinsulinaemia is sufficient to limit the efficacy of agents that target PI3K or AKT, there would be a rationale for combining these agents with kinase inhibitors that specifically target the IIRF. In this context, the main therapy would be the downstream inhibitor, and inhibition of IIRF would be used to block the effect of compensatory hyperinsulinaemia. Apart from the possibility that blocking signalling downstream of the IIRF may lead to increased ligand levels that could limit efficacy, there is evidence that such blockade also increases the expression of receptor tyrosine kinases, including the insulin and IGFI receptors[62], which again raises the possibility of co-targeting.

New agents that refine classic approaches to endocrine therapy of prostate cancer provide interesting precedents for undesirable systemic effects of drugs that are designed to act in neoplastic tissue. The use of an inhibitor of androgen synthesis results in increased gonadotrophin levels that are sufficient to overcome the inhibition of androgen synthesis, unless an additional agent is used to interrupt the compensatory homeostatic response that increases androgen production. When this combination is used, results are superior to simple castration therapy[63]. Although this example is obviously different from PI3K targeting in terms of the pathways involved, the idea of a systemic response that may limit local action is similar. If the analogy proves valid, it will be necessary to use PI3K inhibitors at doses that are sufficient to cause compensatory hyperinsulinaemia, but then interfere with the consequences of this compensation.

There are important details to consider in the use of insulin-related pharmacodynamic assessments of agents that act downstream of the IIRF, such as PI3K inhibitors. These include the identification of calorific intake as a relevant variable, as well as the timing of measurements in relation to meals. Formal glucose-tolerance tests and insulin-tolerance tests would provide the most detailed information. There are important agent-specific considerations in this context. For example, CAL-101 (REF. 64) is an inhibitor specific for PI3Kδ, which is mainly expressed in haematopoietic cells and thus would not be expected to have an important impact on carbohydrate metabolism, even at effective doses. mTOR inhibitors, acting further downstream of the insulin receptor, act at several levels and have more complex effects on whole-organism carbohydrate physiology. Owing to the inhibition of negative intracellular feedback loops by rapamycin and similar mTOR inhibitors, these agents may actually have some insulin-sensitization activity.

Immortal time bias
This can occur in pharmacoepidemiology studies if determination of treatment status is carried out during a time interval that may, for some subjects, also represent part of the follow-up period for clinical end points.

There is evidence of hyperglycaemia induced by these agents, but this may arise partly owing to the inhibition of insulin synthesis by pancreatic β-cells[65].

Although the effects of targeted therapies directed at signalling nodes downstream of the insulin receptor may provide useful pharmacodynamic markers, there is also a concern that metabolic deregulation of carbohydrate metabolism might lead to dose-limiting toxicity. The use of metformin could be effective in attenuating these adverse effects, and owing to its mode of action it would not be expected to interfere with efficacy. On the contrary, it may even enhance efficacy (as discussed below).

IIRF, metformin and biguanides

Metformin is a biguanide that is commonly used in the treatment of type 2 diabetes. Interest in its relevance to oncology was mostly kindled by retrospective pharmacoepidemiological studies that provided evidence for substantially reduced cancer burden (>50% in some studies) in patients with diabetes treated with this agent compared with patients with diabetes on other treatments. These studies must be regarded as hypothesis-generating rather than as definitive, and statistical issues, such as immortal time bias[66], must be considered. However, the multiplicity of reports with similar conclusions certainly justifies further research.

Some of the proposed mechanisms of action of metformin that have been suggested by laboratory studies are summarized in FIG. 3 and a recent review[67]. There is considerable evidence[68] that the fundamental site of action of metformin is in the mitochondria, where it partially inhibits respiratory complex I, leading to reduced oxidative phosphorylation and reduced ATP production. This leads to a cellular ATP deficit and the activation of AMP kinase (AMPK), which is a cellular energy sensor that downregulates cellular processes that consume energy[69]. In hepatocytes, this leads to energy conservation by the inhibition of glucose output to the circulation through gluconeogenesis, which tends to restore hepatocyte ATP levels. Metformin-induced decreases in oxidative phosphorylation may also inhibit gluconeogenesis through AMPK-independent mechanisms[70]. Inhibition of gluconeogenesis lowers blood glucose, which secondarily reduces hyperinsulinaemia. Such an effect is hypothesized to reduce insulin-stimulated neoplastic growth, and is of considerable interest in the context of studies showing that cancer risk is higher[24] or that the prognosis of certain tumours is worse[25,71,72] in patients with higher levels of insulin secretion, as reflected by c-peptide levels. Furthermore, there are several experimental studies demonstrating that metformin can reduce insulin-stimulated tumour growth *in vivo*[19,22]. However, there are important caveats to consider. The antineoplastic activity of metformin may be limited by the fact that it has no significant effect on IGFI levels, despite the fact that it can lower the increased insulin levels that are seen in type 2 diabetes or obesity. The insulin-lowering effect does not operate when baseline insulin levels are normal, and will be irrelevant for the subset of cancers (such as those that have activating mutations of PI3K) that are not insulin sensitive. Although metformin also has inhibitory effects on respiratory complex I that are similar to those observed in hepatocytes in other untransformed and neoplastic cell types[67,73,74], it is unclear to what extent these effects occur in organs other than the liver *in vivo*, as there are important gaps in knowledge concerning pharmacokinetics. It is not yet clear what proportion of tumours expresses sufficient amounts of the cell surface organic cation transporter 1 (OCT1; also known as SLC22A1) that is required for metformin uptake, or whether the drug achieves the concentrations that are required for biological activity in tissues other than the liver[75,76]. A recently described consequence of metformin action on mitochondrial function involves the reduction of reactive oxygen species production sufficiently to decrease the somatic cell mutation rate, which (if confirmed *in vivo*) would have important implications for the inhibition of carcinogenesis[77].

Although many clinical trials of metformin for indications in oncology have already been launched, it is possible that the use of new information concerning predictive host biomarkers (such as hyperinsulinaemia) or tumour biomarkers (such as lack of PI3K mutations and/or expression of proteins required for importing metformin into cells) to identify suitable patient populations may optimize trial design. Metformin dose (or even choice of the most appropriate biguanide) may be influenced by further data concerning pharmacokinetics. Apart from the possibility that biguanides may have single-agent anti-neoplastic activity in certain contexts, there is considerable interest in rational combination therapies involving biguanides (including, for example, combinations with therapies targeting mutated BRAF[78]).

When used with agents that target the IIRF or downstream elements of the PI3K pathway, biguanides may not only attenuate the hyperglycaemia and hyperinsulinaemia that are associated with IGF1R-targeting agents or PI3K inhibitors, but may also contribute to antineoplastic activity. It will be important to design clinical trials in such a way so that the use of metformin does not become an uncontrolled variable in assessing outcomes. It is plausible that PI3K pathway inhibitors at dosages that require metformin to control hyperglycaemia will be the most likely to have antineoplastic activity. Furthermore, it will be interesting to compare the anticipated effects of adding metformin versus adding an IIRF kinase inhibitor to patients being treated with a PI3K inhibitor. Metformin would be expected to reduce hyperglycaemia and hyperinsulinaemia, but not to eliminate the effect of increased insulin levels on the PI3K blockade, if this is present. Conversely, targeting the IIRF would eliminate any effect that hyperinsulinaemia might have in limiting the efficacy of PI3K blockade, but would not reduce insulin or glucose levels.

Finally, it will be important to uncover any special therapeutic opportunities with which there is synthetic lethality between biguanide-induced energy stress in cancer cells and genetic lesions that confer sensitivity to such stress. Experimental tumours that lack LKB1 or p53 seem to be less able to reduce energy consumption to compensate for biguanide-induced energy stress, resulting in severe ATP depletion and necrotic cell death[19,79]. Furthermore, in the context of therapies

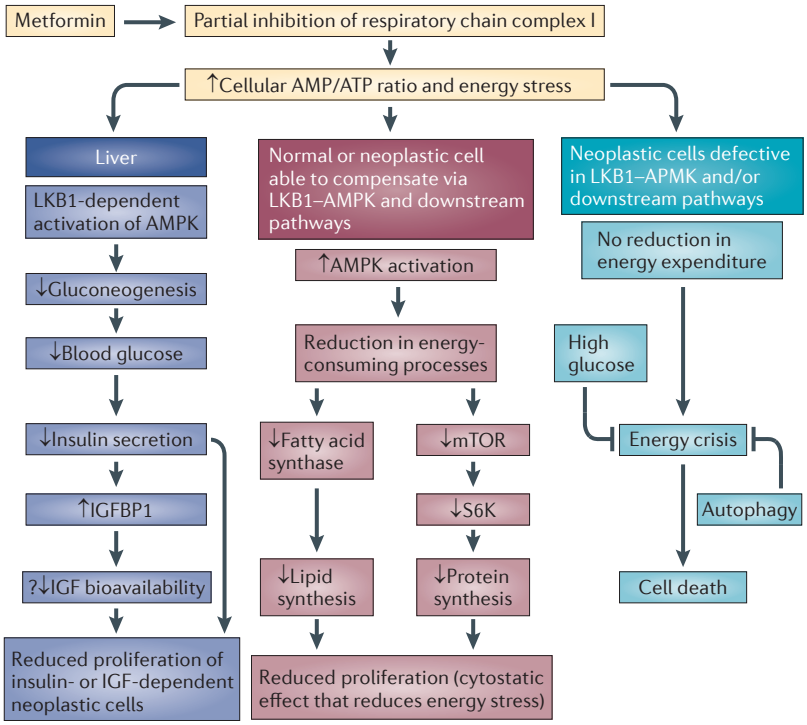

Figure 3 | **Proposed mechanisms of antineoplastic actions of biguanides.** An important classic site of metformin action in type 2 diabetes is the liver. It is known that hepatocytes are exposed to high concentrations of metformin following oral ingestion, and that hepatocytes express cell-surface transport molecules, such as organic cation transporter 1 (OCT1), that are required for drug entry into cells. Metformin probably primarily acts on the mitochondria where it reduces ATP production through the inhibition of respiratory complex I. This leads to the activation of AMPK and to a decrease in gluconeogenesis, which lowers the increased blood glucose, characteristic of type 2 diabetes, with a secondary reduction in hyperinsulinaemia. The reduction in insulin may lead to the less aggressive behaviour of the subset of breast cancers that are insulin-responsive, and also increases concentrations of certain insulin-like growth factor (IGF)-binding proteins, which may reduce IGFI bioactivity, in a way that also would reduce IGF1R activation. Although this indirect mechanism of metformin action does not require any accumulation of the drug in neoplastic tissue, it cannot operate if baseline insulin and glucose levels are normal, as metformin has only minor effects on gluconeogenesis in this situation. It is not clear whether metformin accumulates at sufficient concentrations in neoplastic tissue to have direct effects, and this may vary with the expression of transport proteins by tumours. Biguanides such as phenformin may have better pharmacokinetic profiles than metformin for direct actions on neoplastic tissue. In any case, if biguanides accumulate in cancer cells that have an intact LKB1–AMPK energy stress-sensing system, the reduced ATP generation that occurs secondary to their mitochondrial action will trigger energy-conserving signalling pathways that will result in the inhibition of mTOR and a reduction in processes such as fatty acid synthesis, protein translation and proliferation, resulting in a cytostatic effect. If cancer cells that are defective in energy stress-sensing apparatus are exposed to biguanides, the reduction of mitochondrial ATP production does not result in compensatory reduction in energy expenditure, and an energetic crisis ensues. However, in certain cases, if glucose is present in high concentrations, increased ATP production by glycolysis may be sufficient to compensate for the reduction in oxidative phosphorylation. This suggests that synthetic lethality strategies that target glycolysis at the same time as exposing cells to metformin, might be beneficial, especially if cells have genetic lesions such as loss of function of LKB1 or p53 that render them deficient in sensing or responding to energy stress.

explicitly designed to target cancer energetics[80], there is evidence that cancer cells with biguanide-induced reduction in mitochondrial ATP output become more dependent on glycolysis and sensitized to glycolysis inhibitors[81–83].

IIRF and obesity

There is a relationship between cancer burden and obesity[84]; therefore, the 'obesity epidemic' (REF. 85), particularly in the context of childhood obesity extending into adulthood, threatens to attenuate recent progress in cancer control. The physiological basis for this relationship probably involves the abnormal cytokine and hormonal environment associated with obesity, rather than a direct effect of excess calorific intake (or positive energy balance) on neoplastic tissue. Although many potential mediators have been identified, insulin is an obvious candidate. The insulin resistance that is associated with obesity leads to increased insulin levels, and there is experimental evidence that diets that lead to weight gain and hyperinsulinaemia increase the activation of insulin receptors in neoplastic tissue[19,22]. Exercise, which can attenuate that adverse effect of obesity, lowers insulin levels[86]. Although these data suggest that changes in insulin levels may mediate the effects of energy balance on neoplastic disease, causality has not been formally demonstrated, and other obesity-related metabolic abnormalities may be functionally involved[87]. *In vivo* laboratory models show that positive host energy balance and increased insulin levels are associated with the antiproliferative activity of metformin, which is consistent with the fact that this agent has an effect on high concentrations of insulin, but has little effect on insulin levels when they are normal. It is possible that, in general, patients who are obese and who have hyperinsulinaemia may benefit more from therapies that target the IIRF than patients who are of an ideal body weight. The beneficial effects of calorific restriction may be mediated by reduced levels of insulin, and, if the restriction is severe, reduced level of IGFI as well; the evidence that cancers with activating mutations downstream of the insulin receptor are uninfluenced by calorific restriction[88] is consistent with this view.

IIRF, diabetes and diabetes treatments

The recognition that the expression of insulin receptors is not confined to classic insulin-target tissues such as the liver, muscle and fat, but that it extends to many normal and transformed epithelial tissues raises several questions related to diabetes and its treatment. Patients with type 2 diabetes are known to have modestly increased cancer risk and/or worse cancer prognosis compared with individuals without diabetes (reviewed in REF. 89). This may be at least partly attributable to exposure to abnormally high levels of circulating endogenously produced insulin that is seen in type 2 diabetes. This hyperinsulinaemia is a consequence of attempted compensation for the insulin resistance of classic insulin-target tissues that is characteristic of type 2 diabetes. A hypothetical worst-case scenario would be that cancer cells remain fully insulin-sensitive in this setting, resulting in substantially increased signalling downstream of their insulin receptors. However, the degree to which type 2 diabetes leads to insulin resistance in neoplastic cells, as compared with classic insulin-target tissues, is not well understood. Experimental evidence suggests that diet-induced hyperinsulinaemia is associated with

a modest rather than with a major increase in the growth rate of experimental cancers[18,22,23,90], a finding that raises the possibility that conditions that lead to host insulin resistance may also lead to a degree of insulin resistance in neoplastic cells.

A separate issue is the possibility that insulin therapy for cancer patients with diabetes may increase cancer risk or may worsen cancer prognosis[91]. This is a legitimate concern, particularly as conventional insulin therapy by subcutaneous injection leads to far greater circulating insulin exposure, both in terms of peak concentrations and integrated exposure level over time, than is seen in non-diabetic individuals[92,93]. A specific aspect of this topic is the possibility that certain synthetic insulins may be more dangerous than endogenous insulin from an oncological standpoint. It is plausible that synthetic insulins could interact differently from endogenous insulins with IIRF members that are present on tissues owing to variations in receptor-binding specificity (for example, increased affinity for IGF1R or hybrid receptors, or stronger binding to insulin receptors), or owing to altered pharmacokinetic profiles[94–96]. *In vitro* experiments have shown non-equivalence in receptor binding between various insulins. Clinical and population studies have raised concerns about possible oncological hazards of synthetic insulins but have not demonstrated any conclusively, although minor effects have not been ruled out. This remains an area of controversy[97,98] and active study, as even small differences between insulins, if confirmed, could greatly influence prescribing habits. Careful assessment of the effects of insulin administration through novel routes also requires scrutiny with regard to cancer risks. The possibility of increased lung cancer risk among subjects on a clinical trial of inhaled insulin[99] contributed to the decision by some companies to stop development in this area, and oral insulins under development will require careful preclinical and clinical safety assessments particularly regarding the effects of exposure of gastrointestinal tissue to concentrations higher than normally encountered.

As discussed above, biguanides may have antineoplastic activity, particularly in patients with type 2 diabetes; so, in the case of a cancer patient with type 2 diabetes, where metformin is sufficient to provide glycaemic control, it should be considered as the antidiabetic agent of choice.

What next?

Although the preclinical rationale for targeting the IIRF is strong, clinical trial experience to date has provided evidence against the hypothesis that certain IGF1R-specific antibodies have broad-spectrum activity for unselected patients with advanced cancer. This does not rule out possible activity for other indications, and it is not clear that these results have implications for anti-ligand therapeutic strategies or for broader-spectrum tyrosine kinase inhibitors that address the entire receptor family. It has been pointed out[100] that, in general, clinical trials should take into account the possibility of benefit confined to patient subgroups, and this has not yet been explored in the case of insulin- and IGF1R-targeting therapy. Although no validated predictive biomarkers are available for the next generation of clinical trials, several candidate biomarkers are under investigation and will probably be evaluated as clinical trials proceed. As was the case for several important approved targeted therapies, early experience with IIGF targeting suggests that single agent activity may be insufficient, so the challenge of selecting the most promising combination therapies will have to be addressed. Apart from the issues related to the evaluation of therapies that target insulin and IGFI signalling, this area of research has become relevant to metabolic adverse effects of PI3K inhibitors, AKT inhibitors and mTOR inhibitors, to the relationship of obesity and cancer, and to the safety assessment of therapies used in the treatment of type 2 diabetes.

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