Targeting TGF- β Signaling for Therapeutic Gain

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Transforming growth factor β s (TGF- β s) are closely related ligands that have pleiotropic activity on most cell types of the body. They act through common heterotetrameric TGF- β type II and type I transmembrane dual specificity kinase receptor complexes, and the outcome of signaling is context-dependent. In normal tissue, they serve a role in maintaining homeostasis. In many diseased states, particularly fibrosis and cancer, TGF- β ligands are overexpressed and the outcome of signaling is diverted toward disease progression. There has therefore been a concerted effort to develop drugs that block TGF- β signaling for therapeutic benefit. This review will cover the basics of TGF- β signaling and its biological activities relevant to oncology, present a summary of pharmacological TGF- β blockade strategies, and give an update on preclinical and clinical trials for TGF- β blockade in a variety of solid tumor types.

The three transforming growth factor β s (TGF- β s), TGF- β 1, - β 2, and - β 3, are closely related ligands that act on most cells types of the body and have pleiotropic activities. Expression of TGF- β 1 is activated by tissue perturbations that induce cellular stress, such as cell proliferation and inflammation, and the induced ligand acts to reestablish homeostasis, acting as part of a negative feedback circuit (Cui et al. 1995; Akhurst et al. 1988; Li and Flavell 2008). In many diseased states, however, including fibrosis and cancer, TGF- β expression is chronically and aberrantly elevated (Derynck et al. 1987; Fowlis et al. 1992; Gorsch et al. 1992; Walker and Dearing 1992; Bellone et al. 1999, 2001). What is more, responses to the ligand are altered toward events that promote disease progression (Derynck et al. 2001; Roberts and Wakefield 2003). This is especially true in cancer, in which a mul-

titude of TGF- β -induced tumor promoting effects modulate the tumor cells directly through enhancement of tumor cell invasion and metastasis, and induction and maintenance of cells with tumor initiating properties, sometimes termed cancer stem-like cells (CSCs). Another major site of protumorigenic TGF- β activity is the tumor microenvironment (TME). Here, TGF- β induces extracellular matrix (ECM) deposition, myofibroblast differentiation, and angiogenesis, and suppresses both the innate and adaptive immune systems. This results in a feed-forward circuit of interactions between the tumor and TME, which furthers tumor progression and results in aggressive, invasive, and metastatic tumors that can be desmoplastic, with elevated intratumoral tension and high interstitial fluid pressure (IFP), all features that may be ameliorated by TGF- β signaling blockade. Over

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the last two decades this signaling pathway has therefore become a target for drug development, both for fibrosis and for oncology (Akhurst and Hata 2012). This review will focus on oncology applications, because there has been a rebirth of interest in TGF- β blockade for cancer immunotherapy with the clinical successes in this rapidly expanding field. Drug development for fibrosis applications, other than that related to oncology, is not addressed because this topic has been reviewed previously (Akhurst and Hata 2012). Moreover, treatment of chronic fibrotic conditions by current anti-TGF- β signaling drugs may be challenging because of the need to define a therapeutic window and dosing regimen between efficacy and side effects. This review will cover the basics of TGF- β signaling and its biological activities relevant to oncology, present a summary of pharmacological TGF- β blockade strategies, and give an update on preclinical and clinical trials for TGF- β blockade in a variety of solid tumor types.

THE TGF- β SIGNALING PATHWAY

The TGF- β family is comprised of more than 30 different homo- and heterodimeric pleiotropic ligands encoded by 33 different genes. The family includes TGF- β s, bone morphogenetic proteins (BMPs), GDFs (growth and differentiation factors), activins and inhibins, nodal, and anti-Müllerian hormone (AMH) (Schmierer and Hill 2007). Each of these ligands binds to and activates signaling through heteromeric combinations of dual specificity kinase receptors that phosphorylate and activate downstream Smad and non-Smad signaling components in a receptor kinase-dependent or independent manner (Fig. 1) (Derynck and Zhang 2003; Sorrentino et al. 2008). There can be considerable cross talk between intracellular signaling pathways of the different TGF- β subfamilies, both downstream from and upstream of their respective receptors (Ray et al. 2010; Grönroos et al. 2012; Peterson and O'Connor 2013). In disease

Figure 1. Context-dependent transforming growth factor β (TGF- β) signaling outputs arise from pathway interactions. Schematic of TGF- β signaling via the canonical Smad pathway (*center*) or noncanonical signaling pathways (*right*). Modification of Smad transcriptional output (*left*) may be by posttranslational modification of Smads, such as by linker phosphorylation by extracellular signal-related kinase (ERK) mitogen-activated protein kinase (MAPK) or by the presence or activation status of interacting transcription factors that are regulated by other stress and other growth factor signaling pathways. EGF, Epidermal growth factor; FGF, fibroblast growth factor; TNF, tumor necrosis factor; PI3K, phosphoinositide 3-kinase; JNK, c-Jun amino-terminal kinase.

states, ablation of signaling from one ligand subtype may interfere with the signaling output from others in either a positive or negative manner, with one of the finest examples being found through human genetics (Hatsell et al. 2015). Chronic versus acute inhibition of TGF- β signaling may result in quite different outcomes (Connolly et al. 2011), because negative feedback loops have evolved to keep this important signaling pathway in equilibrium within the cell (Fig. 1).

The three bona fide TGF- β ligands are distinguished from other ligands of this family by extensive sequence homology and their unique ability to bind and signal via the TGF- β receptor type II, T β RII. When the homodimeric ligands bind to the extracellular domain of T β RII, hetero-oligomerization of the two canonical receptors, T β RI and T β RII, causes a conformational change in the receptor complex that results in phosphorylation, and subsequent activation, of the type I receptors by the type II receptors (Shi and Massagué 2003). Activation of T β RI leads to signal propagation through the well-characterized Smad-dependent canonical pathway and/or Smad-independent noncanonical pathway(s) (Derynck and Zhang 2003). Cross talk between Smad and non-Smad signaling pathways can directly modify Smad transcriptional output, as can interaction between TGF- β and other growth factor signaling pathways. Smads, for example, can be phosphorylated within their central “linker” domain by other kinases, such as the extracellular signal-regulated kinase (Erk) mitogen-activated protein kinase (MAPK) pathway (Kretzschmar et al. 1999), c-Jun amino-terminal kinase (JNK) MAPK pathway (Engel et al. 1999), protein kinase C (PKC) (Yakymovych et al. 2001), Erk MAPK 1 (MEKK1) (Brown et al. 1999), and Ca²⁺/calmodulin-dependent protein kinase II (CamKII) (Wicks et al. 2000), which can negatively or positively affect their activity. Thus, the result of ligand stimulation is highly contextual (Fig. 1).

Each of the three TGF- β ligands is translated as a large pre-pro-polypeptide. The amino-terminal signal peptide plays a role in classic intracellular translocation and secretion. The prodomain, otherwise termed the latency-as-

sociated peptide (LAP), is cleaved from the carboxy-terminal mature peptide by a subtilisin-like proprotein convertase, furin, but remains noncovalently associated with the mature homodimeric ligand (Annes et al. 2003). Dimeric LAP encapsulates the mature dimeric peptide within a cage-like structure, keeping it in an inactive state that facilitates spatial and temporal regulation of storage, delivery, and activation (Shi et al. 2011). On the outer surface of this “cage,” one of the two LAP arms also binds covalently to a LTBP (latent TGF- β -binding protein), which is a large ECM component that anchors latent TGF- β within the ECM rather than permitting free diffusion within the tissue (Munger et al. 1997; Ota et al. 2002; Annes et al. 2003). LTBP noncovalently binds fibrillin (Chaudhry et al. 2007), mutations of which are causative for Marfan syndrome (Robinson et al. 2006). Dimeric LAP also binds noncovalently to cell surface integrins, particularly integrins β 1, β 6, and β 8, which can bind latent TGF- β 1 and β 3 via an RGD site within the corresponding LAP domains (Munger et al. 1999; Annes et al. 2004; Yang et al. 2007; Arnold et al. 2014). This interaction results in TGF- β activation, triggered by the molecular tension resulting from physical stretch between LAP-integrin binding at the cell surface and LAP-LTBP anchorage to the ECM (Yang et al. 2007; Shi et al. 2011; Dong et al. 2014; Mi et al. 2015). Proteolytic cleavage of the amino-terminal motif of LAP can also activate TGF- β by releasing the entrapped active homodimer from its latent protein cage (Dong et al. 2014). On the surface of T cells and platelets, the transmembrane protein glycoprotein A repetitions predominant (GARP or LRRC32), a leucine-rich repeat molecule, serves a similar and essential role in activating TGF- β at the cell-platelet surface (Stockis et al. 2009; Tran et al. 2009). Thrombin, matrix metalloproteinases (MMP)-2 and -9, and thrombospondin-1 (THBS1), which can all be enriched in carcinomas, are each capable of cleaving LAP to activate TGF- β (Schultz-Cherry et al. 1994). Other means of ligand activation include acidic pH, reactive oxygen species (ROS) and ionizing radiation, all of which are relevant to tumor progression and cancer

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treatment (Barcellos-Hoff 1993; Barcellos-Hoff and Dix 1996; Annes et al. 2004; Jobling et al. 2006; Shi et al. 2011).

LIGAND EXPRESSION AND TGF- β RESPONSIVENESS IN HUMAN TUMORS

Early studies showed that TGF- β expression levels are elevated in both human and murine tumors relative to their respective normal tissues (Derynck et al. 1987; Fowles et al. 1992; Gorsch et al. 1992; Walker and Dearing 1992; Bellone et al. 1999, 2001). Generally, it is thought that TGF- β 1 and TGF- β 2 are the major players in tumor progression, with only a few reports of TGF- β 3 involvement (Constam et al. 1992). The sparsity of reports of TGF- β 3 involvement in cancer may be because of ascertainment bias (i.e., few studies have systematically examined the expression of all three ligands during tumorigenesis); alternatively, there is disincentive to report negative data (i.e., TGF- β 3 may not be expressed). However, the question of which tissue and what cell types secrete the various ligands is critical when using ligand-specific drugs (Bedinger et al. 2016), especially in the light of older reports that TGF- β 1 and TGF- β 3 may have antagonistic effects (Li et al. 1999; Ask et al. 2008; Lavery et al. 2009). In breast cancer, which has been most extensively studied in this respect, reports suggest that both TGF- β 1 and TGF- β 2 are functionally associated with a more invasive and early onset breast cancer, whereas TGF- β 3 expression appears higher in more differentiated lobular breast tumors (Flanders and Wakefield 2009).

Circulating plasma levels of TGF- β 1 and - β 2, but not TGF- β 3, can be exceedingly high in cancer patients, correlate with tumor grade, and decrease significantly following surgical tumor resection (Kong et al. 1995; Krasagakis et al. 1998; Shim et al. 1999). In particular, TGF- β 1 expression is frequently activated in response to many forms of cellular stress, such as during epithelial hyperplasia, where it serves a function in reestablishing homeostasis (Akhurst et al. 1988; Cui et al. 1995). However, during tumor progression, the normal function of TGF- β as a

homeostatic negative regulator of cellular proliferation is blunted by activation of oncogenes. Moreover, TGF- β 1 expression is sequentially elevated by multiple mechanisms during the progression of multistage carcinogenesis from tumor initiation through to metastasis (Oft et al. 2002)—for example, by oncogene-driven AP-1 transcription factor binding to and activation of the *TGFB1* gene promoter (Kim et al. 1989; Weigert et al. 2000; Davies et al. 2005) and by latent TGF- β activation within a protease-rich TME (Leitelin et al. 2001). Tumor and patient are therefore bathed in excess TGF- β , contributing to an immunosuppressed state.

Which cells within the tumor respond to TGF- β , and how they respond, are highly relevant questions for the design of TGF- β -blockade therapy. Parenchymal cells of certain tumors are unresponsive to TGF- β because of genetic inactivation of *TGFB1* or *TGFB2* or loss of intracellular signaling pathway components (Akhurst and Derynck 2001). This is particularly true for cancers of the gastrointestinal (GI) tract, including colon, pancreas, and gastric cancer. In colon cancer with microsatellite instability (MIS), *TGFB2* is a mutational hotspot for genetic inactivation caused by the possession of a 10-bp polyadenine repeat within its coding sequence (Parsons et al. 1995). However, loss of a single component of the pathway, such as *SMAD4/DPC4* in pancreatic cancer (Iacobuzio-Donahue et al. 2009), does not necessarily ablate all TGF- β responses. Tumor cells are highly plastic, and can rewire signaling networks independently of Smad4 (Hocevar et al. 1999; Giehl et al. 2007; Descargues et al. 2008). Moreover, in GI and other tumor types, such as glioblastoma, prostate cancer, hepatocellular carcinoma, bladder, and breast cancer, mutational loss of TGF- β signaling components is uncommon, whereas transcriptional or epigenetic repression of genes encoding signaling components can occur (Ogino et al. 2007; Shitsin et al. 2007; Yamashita et al. 2008; Dong et al. 2012). A study of 34 matched primary and recurrent breast tumors shows that, despite apparent lack of *TGFB2* mutations in primary tumors, 12% of recurrent breast tumors contain receptor kinase-attenuating point mutations,

suggesting that *TGFBR2* mutation in a minority of breast tumors is a late event rather than a driver (Lucke et al. 2001). In human cutaneous squamous cell carcinoma (cSCC), attenuated expression of Smad proteins has been reported (Hoot et al. 2008), but whether this is owing to mutation or transcriptional or epigenetic dysregulation was not investigated. In head and neck squamous cell carcinoma (HNSCC), mutations in *TGFBR2* and *SMAD4* occur but at low frequency (1% or less) (TCGA Network 2015).

Examination of Smad activation in archival human tumor samples, probed by phospho-Smad (pSmad) immunoreactivity, may be misleading (Xie et al. 2002; Ogino et al. 2007; Hoot et al. 2008; Harradine et al. 2009), not only because of the common limitations of immunohistochemistry (IHC), such as specificity and nonlinear sensitivity, but also because pSmad2 activation is heterogeneous and highly dynamic within the tumor. IHC analysis has shown increased levels of activated nuclear pSmad2 at the invasive front of human breast tumors (Kang et al. 2005), and intravital microscopy using a fluorescent reporter to track Smad activation in live mouse tumors reveals the dynamic nature of this activity within breast cancer cells in vivo (Giampieri et al. 2010). The latter study shows that TGF- β signaling promotes single cell motility and metastasis in a transient manner. Importantly, TGF- β signaling is down-regulated at the destination site of metastasis, favoring outgrowth of secondary tumors (Giampieri et al. 2010). Clearly, active TGF- β signaling depends not only on the tumor genetics but also, in a dynamic fashion, on a cell state that fluctuates between active and inactive signaling. Furthermore, the response to TGF- β signaling also depends on the status of other signaling and transcriptional pathways, which are probably also in dynamic flux (Fig. 1).

Even when the carcinoma cells are resistant to TGF- β signaling by virtue of genetic or epigenetic loss of *TGFBR2*, the cells of the TME retain an intact signaling pathway. These effects of TGF- β on tumor stroma, vessels, and immune cells may turn out to be the Achilles' heel in TGF- β blockade therapy (see below).

Targeting TGF- β Signaling for Therapeutic Gain

TGF- β ACTIONS IN TUMORIGENESIS

Inhibition of Cell Proliferation

TGF- β is a most potent epithelial growth inhibitor, as well as a suppressor of endothelial, hematopoietic, and immune cell proliferation (Coffey et al. 1988; Derynck et al. 2001). The proapoptotic and differentiation-inducing activities of TGF- β on epithelial cells, in the context of cancer, result in tumor suppression (Heldin et al. 2009). In normal epithelial cells, TGF- β activates transcription of *CDKN1A* and *CDKN2A*, which encode the cyclin-dependent kinase (CDK) inhibitors, p21^{CIP1} and p15^{Ink4b} respectively, causing cell-cycle arrest at the G₁ phase (Gomis et al. 2006a). Conversely, TGF- β acting via a Smad–FoxO complex represses the transcription of *MYC* and *ID* family genes, which encode transcription factors that control cell proliferation, cell fate determination, and cellular differentiation (Siegel et al. 2003; Kondo et al. 2004; Tang et al. 2007; Anido et al. 2010; James et al. 2010). In carcinomas, many tumors lose the growth inhibitory response to TGF- β , but still respond to this ligand but in a protumorigenic manner, such as increased migration, invasion, and epithelial–mesenchymal transition (EMT). Thus, depending on the tumor type and the stage of tumor progression, TGF- β may potentially suppress or promote cancer progression through direct actions on the tumor cells (Gomis et al. 2006b; Hannigan et al. 2010), presumably through its control over differential gene expression programs (see below).

Extracellular Matrix Regulation

The ECM is the noncellular component of connective tissue, which supports cells and their functions, and is composed of multiple proteins, collagen, elastin, fibrillin, fibronectin, laminin, and proteoglycans. Fibrosis is characterized by the accumulation and activation of fibroblasts to secrete excessive ECM, and this is stimulated by TGF- β , making this signaling pathway a therapeutic target in various pathological fibroses (Akhurst and Hata 2012). Several genes encoding ECM proteins that are known to be important in driving fibrosis are directly

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activated by TGF- β -induced Smad and Smad-independent signaling (Stampfer et al. 1993; Hocevar et al. 1999, 2005; Piek et al. 2001). The fibrotic response to TGF- β is highly relevant to cancer progression, because a desmoplastic response is seen in many cancer types, especially pancreatic cancers and mesothelioma (Kano et al. 2007; Margadant and Sonnenberg 2010; Fujii et al. 2012a,b). Desmoplasia not only presents a barrier to drug delivery (Kano et al. 2007), but can also provide feedback on the tumor to promote growth and metastasis (Levental et al. 2009; Ng and Brugge 2009). There is reciprocal regulation between TGF- β by ECM. Latent TGF- β bound to ECM components, such as fibronectin and fibrillin, is inactive until physiological or pathological processes initiate its release (Chaudhry et al. 2007; Shi et al. 2011). Moreover, interaction between integrins and ECM can generate tension that feeds back to more TGF- β expression and activation, generating a cycle toward cumulative fibrosis.

Induction of Epithelial–Mesenchymal Transition and the Myofibroblast Phenotype

Partial or full EMT can be induced by TGF- β in both normal and neoplastic epithelia and has consequences for disease progression by stimulating invasion, metastasis and seeding potential of primary tumor cells, as well as contributing to a stromal component that is refractile to drugs (Derynck and Akhurst 2007). Expression of E-cadherin, which can suppress tumor progression, is commonly down-regulated within many carcinomas during EMT (Caulin et al. 1995; Portella et al. 1998; Lacher et al. 2006; Fransvea et al. 2008; Hoot et al. 2008). The TGF- β -Smad pathway mediates expression of high-mobility group A2 (HMGA2), which contributes to the induction of the expression of Snail and Slug, two zinc-finger transcription factors that repress the E-cadherin gene (Padua and Massagué 2009). In breast and skin cancer, tumor cell EMT contributes to cancer progression, as cells consequently become more migratory, invasive, and can ultimately transition to a myofibroblastic phenotype (Derynck and Akhurst 2007). Myofibroblasts further modu-

late the basic biology of the tumor by increasing ECM elaboration and eliciting a tissue contraction process, which contributes to further TGF- β activation (Shi et al. 2011) and to elevation in IFP (Lammerts et al. 2002; Heldin et al. 2004; Salnikov et al. 2005). This attenuates the efficiency of delivery of some drugs to the tumor, because, under positive IFP, small molecule drugs do not efficiently penetrate tumor tissue (Heldin et al. 2004). Conversely, the leaky nature of tumor vasculature presents an opportunity for drug delivery of novel nanoparticles to the tumor (Kano et al. 2007; Park et al. 2012), which is enhanced by T β RI blockade (Kano et al. 2007).

TGF- β Action in Cancer Stem-Cell Maintenance

Accumulating evidence links TGF- β signaling to the acquisition and maintenance of a “stem-cell-like” state of carcinoma cells. The elusive tumor-initiating cell or CSC generates diverse progeny that lead to the characteristic heterogeneity of the tumor while also maintaining a self-propagating function that is essential for primary tumor growth and for seeding of tumors at distant metastases (Shipitsin et al. 2007; Mani et al. 2008). It is now widely accepted that EMT can generate a CSC-like state; indeed, this cell type is reported to occupy a partial EMT position with characteristics of both cell states (Kalluri and Weinberg 2009; Jolly et al. 2015).

There is enrichment of expression of TGF- β signaling components in CD44⁺/CD24[−] breast carcinoma cells with CSC-like properties relative to their CD44[−]/CD24⁺ progeny (Shipitsin et al. 2007). CD44⁺ cells, but not their progeny, express *TGFB1* and *TGFBR2* together with a “TGF- β gene expression signature.” Indeed, within the non-CSC compartment, epigenetic modifications were found that silence the *TGFBR2* gene (Shipitsin et al. 2007). Short-term treatment with LY2109761, a small molecule inhibitor of the T β RI and T β RII kinases, (Sawyer et al. 2003; Yingling et al. 2004) decreases the expression of markers of “stemness” and induces differentiation to a less aggressive

carcinoma type, suggesting that TGF- β signaling controls stem-cell maintenance (Shipitsin et al. 2007). In support of this postulate, decreased expression of CSC markers was also detected in tumor cells of a single glioblastoma patient undergoing the first clinical trial of the small molecule T β RI inhibitor, galunisertib (Anido et al. 2010). However, others made contrary findings in gastric carcinoma cells in culture and in a breast carcinoma xenograft model (Tang et al. 2007; Ehata et al. 2011). T β RI–Smad2 signaling has been implicated in maintaining epigenetic silencing that promotes and sustains EMT and the CSC phenotype (Papa-georgis et al. 2010). Even in hematological malignancies, in which TGF- β has a predominantly tumor suppressive role, TGF- β signaling supports leukemia-initiating cell maintenance in chronic myeloid leukemia (CML) through its association with the FOXO pathway and activation of Akt signaling (Naka et al. 2010; Miyazono 2012). It has also been shown that hemangioblasts from BCR-ABL-positive CML patients express higher levels of TGF- β 1 (although not TGF- β 2 or TGF- β 3) than those from control individuals. This was attributed to activation of TGF- β 1 by the BCR-ABL oncoprotein (Zhu et al. 2011). Indeed, the inhibitor of ABL tyrosine kinase, imatinib, suppresses the expression of TGF- β 1 by CML hemangioblasts. Conversely, treatment of CML hemangioblasts with TGF- β 1 increases the expression of MMP9, soluble KitL and soluble ICAM-1, which all contribute to CML progression. Moreover, TGF- β induces ICAM-1 synthesis and suppresses the activity of T lymphocytes and natural killer (NK) cells. BCR-ABL-induced TGF- β 1 not only assists stem-cell maintenance, but produces an immune privileged microenvironment for the stem cells (Zhu et al. 2011). TGF- β inhibitors might therefore be uniquely poised to kill or maim CSCs, making this class of drug even more attractive to oncologists.

TGF- β Signaling Promotes Resistance to Chemotherapy

EMT and the resultant CSC-like phenotype not only help drive metastasis, but also contribute

to chemotherapeutic drug resistance (Singh and Settleman 2010; Huang et al. 2012). Some tumors show intrinsic resistance to chemotherapy while others acquire resistance after prolonged exposure to drug (Engelman and Settleman 2008; Sequist et al. 2008; Corcoran et al. 2010). The unpleasant truth in oncology is that in the most aggressive tumor types, such as melanoma, lung, pancreas, and glioblastoma multiforme, patients may initially respond well to chemotherapy but then have an exceedingly high rate of relapse as a consequence of “acquired” drug resistance. Similarly, even with the newer wave of targeted therapies, such as herceptin (anti-HER2 mAb) and vemurafinib (small molecule B-Raf inhibitor), drug resistance develops within months of treatment. The mechanisms for development of drug resistance are varied, with some targeted therapies evolving highly specific mechanisms to avoid drug action. With epidermal growth factor receptor (EGFR) inhibitors in non-small-cell lung carcinoma (NSCLC) therapy, ~50% to 70% of cases of acquired drug resistance involve somatic mutation of the gene encoding the drug target, namely *EGFR*. In particular, a common T790M “gatekeeper” mutation changes the relative binding of EGFR to the ATP-mimetic drug versus its binding to endogenous substrate, ATP (Yun et al. 2008). Amplification of the *MET* oncogene is another common cause of drug resistance (Engelman et al. 2007). However, in 30% to 50% of cases, there is no specific drug-evading mutation. Instead, global epigenetic changes take place that lead to an altered chromatin state (Sharma et al. 2010; Vinogradova et al. 2016). This altered cellular differentiation state is likely reached during EMT, and coincides with the appearance of chemoresistant cells with stem-cell-like features, coined drug-tolerant persister cells (DPTs) (Voulgari and Pintzas 2009; Sharma et al. 2010). Even in breast carcinomas driven by *HER2/NEU/ERBB2* amplification, epithelial-like luminal tumors evolve to enrich for CD44^{high}CD24^{low} cells with the characteristics of CSCs, and these tumor cells are resistant to neoadjuvant chemotherapy and pharmacological HER2 inhibition (Li et al. 2008). Generally, although patients

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with basal mesenchymal-like tumors may initially respond to chemotherapy better than those with luminal breast cancer, individuals with triple negative breast tumors ultimately have the worst prognoses, possibly because of a more aggressive CSC-like subpopulation that is drug resistant.

The central importance of TGF- β signaling in driving a drug tolerant state has been illustrated by the discovery of a suppressor of T β RII expression in a genome-wide RNAi screen to identify genes that mediate chemoresistance of breast cancer cells (Huang et al. 2012). Intriguingly, up-regulation and enhanced cell surface presentation of T β RII protein is mediated by loss of the *MED12* gene (encoding a subunit of the mediator complex), and this was the most common cause of drug resistance in these breast carcinoma cells. Indeed, up-regulation of T β RII expression appears to be a common contributor to acquired resistance against numerous drugs, including chemotherapeutics, such as cisplatin, as well as the molecular targeted therapies, erlotinib and gefitinib (EGFR inhibitors), sorafenib (a multikinase RAS/RAF/ERK, VEGFR, PDGFR inhibitor), PLX4032 (a BRAF inhibitor), and crizotinib (a MET and ALK tyrosine kinase inhibitor). Moreover, as a mechanism of acquired drug resistance, up-regulation of T β RII consequent to loss of MED12 is observed in a variety of different tumor types, including NSCLC, colorectal cancer, melanoma, and hepatocellular carcinoma. The increased expression of T β RII results in activation of both Smad and non-Smad Erk MAPK signaling in response to autocrine TGF- β , and is both sufficient and necessary for acquired cancer drug-resistance (Huang et al. 2012). Importantly, the small molecule T β RI inhibitor, LY2157299 (galunisertib), resensitizes drug-tolerant cells to anticancer drugs (Huang et al. 2012). Proof of this concept was shown in clinical material from paclitaxel-treated primary breast cancer patients wherein paclitaxel treatment in the neoadjuvant setting increased a TGF- β gene expression signature in the primary tumor (Bhola et al. 2013). *TGFBR2*, *TGFBR3*, and *SMAD4* mRNA levels were increased approximately twofold, *TGFBR1*, *TGFB1*,

TGFB3, *SMAD2*, and *SMAD7* to a lesser degree, and the CSC-related *CD44* and *ALDH1* mRNAs were also elevated after chemotherapy. Moreover, coadministration of paclitaxel with TGF- β signaling inhibitors (galunisertib, anti-T β RII antibody or *siSMAD4*) in a nude mouse xenograft model reduces primary tumor growth compared with tumors receiving paclitaxel alone (Bhola et al. 2013). Most important, the number of tumor initiating cells within the primary tumor, assayed by their ability to generate mammospheres in cell culture, was decreased in mice treated with the TGF- β inhibitor compared with paclitaxel alone (Bhola et al. 2013). Because the tumor initiating cells are essential for tumor growth and metastasis, these studies show the essential need for TGF- β signaling for tumor growth and metastatic spread.

In a model of cSCC, TGF- β contributes to drug resistance by yet another mechanism, independent of its effect on cell cycle or DPTs. Specifically, induction of p21^{Cip1} expression in response to TGF- β activates the expression of the transcription factor Nrf2, which in turn activates genes of the glutathione-S-transferase pathway. Activation of this pathway is thought to neutralize tumor-damaging ROS, generated consequent to chemotherapy (Oshimori et al. 2015).

TGF- β as a Potent Suppressor of Tumor Immunosurveillance

The negative regulation of the immune system by TGF- β is complex and context-dependent, but was recognized as a target for cancer therapy as early as 1988 (Kasid et al. 1988; Kuppnier et al. 1988; Zuber et al. 1988), and proof-of-principle was shown in vivo in 1993 (Arteaga et al. 1993; Gridley et al. 1993). Under normal conditions, TGF- β signaling delicately regulates the tolerogenic versus immunogenic arms of the immune system to balance an adequate host defense to foreign substances while limiting collateral inflammatory tissue damage (Gorelik and Flavell 2001, 2002; Rubtsov and Rudensky 2007; Flavell et al. 2010). TGF- β has potent growth suppressing activity on most precursor cells of the immune system, particularly T and B cells of the adaptive arm. It suppresses T-cell proliferation

(Rubtsov and Rudensky 2007), induces B-cell apoptosis (Ramesh et al. 2009), and suppresses the effector functions of cytotoxic CD8 T cells and helper CD4⁺ T cells (Gorelik and Flavell 2002). Regulatory T cells (Tregs) that suppress the activity of effector T cells, are stimulated to expand and differentiate by TGF- β , and Treg functionality is mediated in part by further TGF- β secretion (Chen et al. 2005).

TGF- β signaling also has potent immunosuppressive action via direct effects on innate immune cells, which modulate their phenotype from tumor cell destroying to tumor cell supporting in response to this cytokine (Mantovani et al. 2002; Fridlender et al. 2009). TGF- β suppresses the generation of NK cells in response to interferon- γ , which is required for NK tumor killing activity, through transcriptional control of the interferon- γ promoter by Smad3 (Laouar et al. 2005). It also “polarizes” macrophages (Mantovani et al. 2002) and neutrophils (Fridlender et al. 2009) from an inflammatory phenotype that targets and destroys foreign agents, such as cancer cells, toward an immunosuppressive protumorigenic cell type (Flavell et al. 2010). Dendritic cells (DCs) show reduced antigen presentation capability in the presence of TGF- β (Yamaguchi et al. 1997), and tumor-derived TGF- β also alters chemokine receptor expression to blunt DC chemotaxis (Sato et al. 2000), further suppressing immune surveillance, which can be relieved by small molecule T β RI kinase inhibitors.

POTENTIAL ONCOLOGY APPLICATIONS FOR TGF- β BLOCKADE

Many solid tumor types share similar properties, regardless of their site of origin. For example, breast cancer, prostate cancer, and melanoma metastasize to bone, and multiple myeloma also colonizes bone. One would expect a therapeutic benefit from TGF- β therapy in these bone-tropic cancers (Yin et al. 1999; Kang et al. 2003; Kozlow and Guise 2005; Mohammad et al. 2011), and targeting of all three isoforms, or at least TGF- β 1 and TGF- β 2, should help neutralize the TGF- β -enriched microenvironment of the bone. This approach might reduce

the osteolytic effects of these cancers, and help reestablish a homeostatic equilibrium that neutralizes bone loss and encourages normal bone growth. Therefore, when considering potential disease indications for anti-TGF- β therapy, the organ site of neoplastic origin should not be the primary consideration. Rather, it is necessary to consider (1) the genetic makeup of the tumor (e.g., mutations in *TGFBR2*, *TGFBR1*, *SMAD4*), (2) activation of a TGF- β responsive gene expression signature, (3) extent of excess TGF- β production (TGF- β 1 or TGF- β 2) by the tumor or its environment, as measured in the circulation or from tumor biopsy, (4) tumor stage and grade, invasive or metastatic, (5) specificity of metastatic tropism (e.g., to bone that is rich in TGF- β), and (6) host cellular responses to the tumor (e.g., local or systemic levels of Tregs or Th17 cells) or desmoplastic responses.

Based on preclinical studies and clinical trials, it is unlikely that TGF- β inhibitors will be efficacious when used alone, because they are not cytotoxic. Moreover long-term treatment regimens, as with any drug, should be avoided because of potential inflammatory, autoimmune, and cardiovascular side effects (Laping et al. 2007; Anderton et al. 2011). It is likely that TGF- β inhibitors will have their most important therapeutic activity in cancer through effects on the TME, particularly, but not only, in neutralizing or reversing immune suppression. Indeed, the combination of TGF- β inhibition together with existing immunotherapies, such as cancer vaccines (Jia et al. 2005; Nemunaitis and Murray 2006; Kim et al. 2008), adoptive T-cell transfer (Suzuki et al. 2004; Wallace et al. 2008), chimeric antigen receptor T-cell therapy (Gill and June 2015; Wu et al. 2015), and immune checkpoint blockade (Topalian et al. 2012; Lipson et al. 2013), will likely provide the major basis of their therapeutic usage. Here again, the major players appear to be TGF- β 1 and - β 2. Augmenting adoptive T-cell therapy with short-term acting drugs, rather than genetic manipulation of tumor homing cytotoxic T lymphocytes (CTLs) to reduce their sensitivity to TGF- β , may be a particularly attractive application because patients need not be exposed to genetically manipulated T cells.

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Moreover, patients may avoid potential side effects of long-term systemic drug exposure as only short-term exposure to the drug may be required for efficacy.

DRUGS THAT BLOCK TGF- β SIGNALING

Drugs that target many different components of the canonical TGF- β signaling pathway have been developed (Fig. 2; Table 1), including a pyrrole-imidazole polyamide drug that blocks transcription of the *TGFB1* target gene (Yao et al. 2009; Chen et al. 2010; Matsuda et al. 2011; Washio et al. 2011; Igarashi et al. 2015), antisense RNAs that target *TGFB1* or *TGFB2* mRNAs for degradation (Hau et al. 2009; Vallieres 2009; Schlingensiepen et al. 2011), antibodies against TGF- β ligands or receptors that block ligand–receptor engagement, and many small molecule ATP-mimetic T β RI kinase inhibitors (Fig. 2). Each drug has distinct advantages and disadvantages that have to be balanced

in assessing their potential for use in the clinic. Parameters to consider are affinity and specificity for drug target, drug stability, drug clearance and bioavailability in vivo, as well as mode of drug delivery (e.g., oral vs. intravenous).

Generally, the small molecule kinase inhibitors (SMIs) lack absolute specificity, and, at certain doses, also target activin, nodal, and possibly the myostatin signaling pathways. Moreover, because these inhibitors block T β RI kinase activity, they will not prevent signaling independent of the kinase activity, such as TRAF6-p38 MAPK signaling (Hoccar et al. 2005; Gudey et al. 2014; Sundar et al. 2015). SMIs have poor pharmacokinetics and are generally cleared from the body with a $t_{1/2}$ of ~ 2 – 3 h, whereas pharmacodynamic markers, such as inhibition of Smad2 phosphorylation, persist for up to 8 h postdosing (Bueno et al. 2008; Gueorguieva et al. 2014). These negative features of SMIs are balanced by the ease of drug administration through the oral route. In some cases, the short pharmacokinetic/pharmacody-

Figure 2. Drug targets on the TGF- β signaling pathway. The figure indicates molecular targets during the synthesis, activation, and signaling of TGF- β to which drugs have been raised. Molecular targets and processes are shown in boxes.

Targeting TGF- β Signaling for Therapeutic Gain**Table 1.** Summary of TGF- β signaling blockade drugs currently in development as clinical leads

Drug class	Drug name	Target	Application	References
Small molecule kinase inhibitors	TEW-7197	T β RI	Oncology	Jin et al. 2014; Son et al. 2014; Naka et al. 2016
	Galunisertib (LY2157299)	T β RI	Oncology	Fujiwara et al. 2015; Kovacs et al. 2015; Rodón et al. 2015b; Brandes et al. 2016
Anti-TGF- β ligand antibodies	Fresolimumab	TGF- β 3>TGF- β 2>TGF- β 1	Oncology, fibrosis	Morris et al. 2014 Rice et al. 2015
	XPA681	Pan-TGF β (β 1> β 2> β 3)	Eye diseases	Bedinger et al. 2016
	XPA089	TGF- β 1>TGF- β 2 no reactivity to TGF- β 3	Oncology	
	LY2382770	TGF- β 1-specific	Fibrosis, oncology	Cohn et al. 2014
Anti-T β R receptor antibodies	LY3022859	T β RII	Oncology	Zhong et al. 2010
Antisense oligonucleotides	ISTH0036, ISTH0047	<i>TGFB2</i> RNA	Eye diseases Preclinical oncology	Bogdahn et al. 2011; Chamberlain 2011; Wick and Weber 2011
Pyrrole-imidazole polyamides		<i>TGFB1</i> gene promoter	Fibrosis, scarring, vascular repair	Yao et al. 2009; Chen et al. 2010; Matsuda et al. 2011; Washio et al. 2011; Igarashi et al. 2015

namical (PK/PD) window may, in fact, be advantageous to minimize side effects. “Off-target” inhibition of activin, nodal, or even p38 MAPK signaling may also enhance drug efficacy, albeit not with the specificity intended. It has been postulated that SMIs penetrate tumor tissue more easily than antibodies, and this may be true in highly dysplastic tumors, such as pancreatic carcinoma. However, the anti-TGF- β antibody, fresolimumab, was shown to be efficaciously delivered to glioblastoma cells in a clinical setting, presumably owing to breakdown of the blood brain barrier in this neoplastic tissue (Den Hollander et al. 2015).

Many preclinical studies have been undertaken, thus evaluating the entire spectrum of T β RI inhibitor drugs (Akhurst and Hata 2012), but the main SMI drug to proceed to the clinic has been galunisertib or LY2157299 (Eli Lilly) (Table 2). More recently, a first human dose (FHD) study of a new T β RI kinase SMI, TEW-7197 (MedPacto), was started as monotherapy in subjects with advanced stage solid

tumors (all cancers, NCT02160106) (Jin et al. 2014; Son et al. 2014; Naka et al. 2016). TEW-7197 has been shown to cause Smad4 degradation in cytotoxic T cells, resulting in enhanced cytotoxic T-cell activity (Yoon et al. 2013), as well as reduced breast tumor metastasis to lung in mice (Son et al. 2014).

The other drug class that has received considerable attention in the clinic (Table 2) is the TGF- β ligand- and receptor-blocking antibodies, including the pan-TGF- β 1/2/3 blocking antibody, fresolimumab (Genzyme/Sanofi) (Morris et al. 2014), the TGF- β 1-specific blocking antibody LY2382770 (Eli Lilly) (Cohn et al. 2014; Tampe and Zeisberg 2014), and the T β RII blocking antibody LY3022859, also called IMC-TR1 (Eli Lilly, NCT01646203) (Zhong et al. 2010). Other companies followed their lead with the development of alternative TGF- β ligand blocking antibodies, such as an anti-TGF- β 1/2 antibody that is not cross reactive with TGF- β 3, developed by Xoma/Novartis (Bedinger et al. 2016).

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Table 2. Interventional clinical oncology trials of TGF- β signaling blockade

Drug	Target disease	Organ site	Status	Clinical trial number	References
Galunisertib	Phase Ib/II study with gemcitabine and LY2157299 for patients with metastatic cancer (phase Ib) and advanced or metastatic unresectable pancreatic cancer (phase II)	All metastatic tumors and unresectable pancreatic	Completed Ongoing	NCT02154646 NCT01373164	Rodón et al. 2015
Galunisertib	Phase I dose-escalation study of LY2157299 monotherapy in patients with solid tumors (Japan)	All solid tumors	Completed	NCT01722825	Fujiwara et al. 2015
Galunisertib	Randomized phase II study LY2157299 monotherapy versus sorafenib monotherapy versus combination of both drugs in patients with advanced hepatocellular carcinoma	HCC	Ongoing	NCT02178358 NCT01246986	
Galunisertib	Phase II/III study of LY2157299 monotherapy in very low-, low-, and intermediate-risk patients with myelodysplastic syndromes	MDS	Ongoing	NCT02008318	
Galunisertib	Phase Ib/IIa study combining LY2157299 with standard temozolomide-based radiochemotherapy in patients with newly diagnosed malignant glioma	Glioma	Ongoing	NCT01220271	Sepulveda-Sánchez et al. 2015
Galunisertib	Phase I/II LY2157299 monotherapy or lomustine monotherapy versus combination of both drugs in recurrent glioma/glioblastoma	Glioma	Ongoing phase I Ongoing phase II	NCT01682187 NCT01582269	Brandes et al. 2016
Galunisertib	LY2157299 and radiotherapy in metastatic breast cancer	Metastatic breast	Recruiting	NCT02538471	
Galunisertib	Phase Ib trial of LY2157299 with paclitaxel in patients with triple negative metastatic breast cancer	Triple negative metastatic breast	Recruiting	NCT02672475	
Galunisertib	Phase Ib dose-escalation and cohort-expansion study galunisertib in combination with the anti-PDL-1 antibody durvalumab (medi4736) in recurrent or refractory metastatic pancreatic cancer	Metastatic pancreas cancer	Not yet recruiting	NCT02734160	
Galunisertib	Phase Ib/II galunisertib with anti-PD-1 (nivolumab) in advanced refractory solid tumors (phase Ib) and recurrent or refractory non-small-cell lung cancer, hepatocellular carcinoma, or glioblastoma	Refractory solid tumors; recurrent NSCLC, HCC, and glioblastoma	Recruiting	NCT02423343	

Continued

Table 2. Continued

Drug	Target disease	Organ site	Status	Clinical trial number	References
Galunisertib	Study of LY2157299 immunomodulatory activity in patients with solid tumors		Recruiting	NCT02304419	
Galunisertib	LY2157299 and enzalutamide in metastatic castration-resistant prostate cancer	Prostate cancer	Recruiting	NCT02452008	
TEW-7197	First-in-human dose-escalation study of TEW-7197 monotherapy in subjects with advanced stage solid tumors	Advanced stage solid tumors	Recruiting	NCT02160106	
Galunisertib	Single-dose study to evaluate exposure–response relationship between LY2157299 and cardiac qt interval in healthy Japanese and non-Japanese subjects	Healthy patients	Recruiting	NCT02752919	
Fresolimumab	Phase I study of fresolimumab to treat advanced melanoma and renal cell carcinoma	Advanced melanoma and RCC	Completed	NCT00923169	Morris et al. 2008, 2014
Fresolimumab	Guiding fresolimumab treatment of primary brain tumors by ^{89}Zr -fresolimumab PET imaging	Gliomas	Completed	NCT01472731	Den Hollander et al. 2015
Fresolimumab	A phase II trial of fresolimumab in relapsed malignant pleural mesothelioma	Relapsed malignant pleural mesothelioma	Completed	NCT01112293	
Fresolimumab	Safety and efficacy of fresolimumab and localized radiotherapy in metastatic breast cancer	Metastatic breast cancer	Active—not recruiting	NCT01401062	
Fresolimumab	Stereotactic ablative radiotherapy and fresolimumab in phase I/II trial for patients with stage IA-IB NSCLC	NSCLC	Not yet recruiting	NCT02581787	
IMC-TR1 (anti-T β RII antibody; LY3022859)	Phase I study of anti-T β RII monoclonal antibody LY3022859 in patients with advanced solid tumors that failed standard therapy	Advanced solid tumors that failed other therapies	Completed	NCT01646203	Cohn et al. 2014
NIS793	Phase I/Ib study of NIS793 in combination with PDR001 patients with advanced malignancies	Breast, lung, hepatocellular, prostate, renal cancer	Not yet recruiting	NCT02947165	

HCC, Hepatocellular carcinoma; MDS, myelodysplastic syndrome; NSCLC, non-small-cell lung cancer; RCC, renal cell carcinoma.

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MONITORING AND PREVENTION OF TGF- β BLOCKADE-INDUCED DRUG TOXICITIES

On moving TGF- β blockade drugs into the clinic, researchers used considerable caution because of the early findings of the duplicitous activity of TGF- β as either tumor promoter or tumor suppressor during neoplastic progression. Moreover, the finding of severe vascular and/or inflammatory defects in mice with silenced TGF- β 1 expression (Shull et al. 1992; Kulkarni et al. 1993; Dickson et al. 1995) raised additional concerns about the potential for undesirable, possibly even life-threatening, side effects of targeting TGF- β signaling in humans. For this reason, the transition into clinical trials and the patient accrual for trials proceeded slowly. Even once commenced, the clinical trials had long-term holds because of disquieting findings of hemorrhagic lesions within the heart valves, as well as aortic aneurysms in rats and dogs after long-term continuous exposure to the highest levels of TGF- β blockade (Frazier et al. 2007; Laping et al. 2007; Anderson et al. 2011). During hold of the clinical trial, considerable effort was invested in developing biomarkers of drug response and undertaking PK/PD modeling to define a better therapeutic window for drug response (Gueorgieva et al. 2014). Phase I and II studies for galunisertib subsequently incorporated careful monitoring of possible adverse cardiac events using circulating biomarkers, such as troponin I, brain natriuretic protein (BNP) and high-sensitivity C-reactive protein (hs-CRP), echocardiography Doppler imaging for possible mitral and tricuspid valve regurgitation, and screening for potential aneurysms of the ascending aorta and aortic arch by computer tomography (CT) or magnetic resonance imaging (MRI) (Fujiwara et al. 2015; Kovacs et al. 2015; Rodón et al. 2015b; Brandes et al. 2016). In a clinical safety study (Kovacs et al. 2015), only one of 79 patients treated showed an increase in cardiopathologic grade, and then only from a baseline of normal to a mild pathology, as assessed by Doppler. This patient was one of 13 receiving continuous administration of the highest drug dose for >6 mo, and

had no other signs of cardiovascular disease. Galunisertib was therefore concluded to be relatively safe in humans, especially compared with cardiotoxicities of other cancer drugs (Benvenuto et al. 2003). On the basis of these findings, clinical trials were resumed incorporating an intermittent dosing schedule (2 weeks on/2 weeks off).

Many have feared the de novo appearance of unrelated neoplasms or the enhanced outgrowth of the primary tumor in response to TGF- β signaling blockade. Epithelial hyperplasia has been seen in some animal models, including tumor-promoting effects of LY2109761 in renal cell carcinoma (RCC) (Laping et al. 2007). There have also been preclinical reports of the ability of TGF- β blockade drugs to awaken dormant metastatic breast tumor cells within the bone marrow, with the supposition that TGF- β 2 plays a major role in breast tumor cell dormancy (Bragado et al. 2013). During a clinical trial of the pan anti-TGF- β antibody, fresolimumab, for the treatment of end-stage drug-refractory metastatic melanoma and RCC, grade 1 or 2 skin rashes that improved or resolved by the end of study were reported in 10 of 29 patients, and nonmalignant keratoacanthomas (KA) appeared de novo in four of these patients (Morris et al. 2014), whereas a low-grade SCC appeared in another individual (Lacouture et al. 2015). Notably, KAs are commonly seen in response to other targeted therapies such as consequent to treatment with the multikinase inhibitor, sorafenib, or the B-Raf enzyme inhibitor, vemurafenib (Arnault et al. 2012; Lacouture et al. 2012), and these KAs are considered a manageable side effect of cancer therapy. As yet, there have been no published clinical reports of promotion of other cancer types, although this might be because clinical studies to date have focused on severely ill patients who did not survive long enough for such an event to become apparent. We now know that the TGF- β signaling pathway is not the only Janus-faced drug target, because many oncogenic proteins and tumor suppressor gene products, including c-Myc, Ras, and SnoN, have both pro- or antitumor activity (Zhang et al. 2001; Dang et al. 2005; Lamouille

and Derynck 2009). Furthermore, as cancer survivorship improves, it is becoming increasingly apparent that standard of care radiotherapy and chemotherapy can lead to later secondary neoplasms unrelated to the primary tumor (Koo et al. 2015). The goal of eradicating a primary or metastatic tumor, without immediately killing the host or causing longer term negative outcomes remains a major challenge in oncology.

UPDATE ON INTERVENTIONAL CLINICAL ONCOLOGY TRIALS WITH TGF- β BLOCKADE

Antisense RNA Approaches

The first clinical trials targeting the TGF- β pathway for cancer treatment were designed to enhance the immune system of patients with NSCLC using a genetically modified tumor vaccine. The irradiated, and therefore nonproliferative vaccine, belagenpumatucel-L (Lucanix), is composed of four different allogeneic human NSCLC cell lines transfected with a gene encoding antisense *TGFB2*. When injected into the patient, Lucanix promotes an antihost NSCLC cytotoxic T cell response, and vaccine immunogenicity is postulated to be potentiated by reduced TGF- β 2 production. Phase I and II trials went well, the drug was found to be safe, and the phase II results suggested some therapeutic benefit (Nemunaitis et al. 2006, 2009). However, a phase III trial to examine benefit as a NSCLC maintenance therapy following primary treatment (270 Lucanix-treated vs. 262 placebo-treated NSCLC patients) did not meet its primary endpoint criterion of increased overall survival (OS) (Giaccone et al. 2015). Stratification of the data revealed a marginal but significant therapeutic benefit for NSCLC patients who started Lucanix within 12 weeks of their initial chemotherapy (166 drug-treated vs. 149 control NSCLC patients), and in those receiving prior radiation therapy (RT), which might boost tumor antigenicity (median survival 28.4 mo for RT with belagenpumatucel-L treatment vs. 16.0 mo for RT plus placebo; HR 0.61, $p = 0.032$) (Giaccone et al. 2015). Nevertheless,

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the drug is currently not under further clinical development. Antisense TGF- β 2 oligonucleotides have also been under clinical development for the treatment of glioblastoma (Isarna Therapeutics). Although early trials looked promising (Bogdahn et al. 2011), as with all antisense approaches to date, systemic drug delivery has been an issue, with the need for localized continuous delivery directly into the brain tumor by catheter and pump. A next-generation TGF- β 2-selective antisense oligonucleotide, ISTH0047, has entered clinical trial for ophthalmology applications (NCT02406833), and the oncology pipeline remains in preclinical stages.

Therapeutic Antibodies

Two phase I trials of fresolimumab monotherapy have been completed, one focusing on metastatic melanoma (Morris et al. 2014) and another on high-grade glioblastoma patients (Den Hollander et al. 2015). Fresolimumab is a high-affinity fully humanized monoclonal antibody that neutralizes the active forms of human TGF- β 1, TGF- β 2, and TGF- β 3 (K_D s of 2.3 nM, 2.8 nM, and 1.3 nM, respectively) (Rice et al. 2015). The drug has anti-TGF- β activity in humans, as assessed by biomarker analysis (Rice et al. 2015), and was found to be safe, albeit there was de novo appearance of cutaneous benign KAs and one SCC in melanoma patients, when used at the highest drug dosing level (15 mg/kg) (Morris et al. 2014). A phase I trial of fresolimumab for treatment of systemic sclerosis using much lower doses than for oncology studies (1–5 mg/kg), showed unprecedented resolution of skin disease, although most patients developed anemia consequent to GI bleeding (not considered a serious adverse effect), which was most likely specific to complications of their disease rather than the drug alone (Rice et al. 2015). Clinical studies of glioblastoma uptake of ^{89}Zr -labeled fresolimumab after intravenous delivery indicated efficient delivery of the drug into this brain tumor, which appeared to be TGF- β -dependent, because all glioblastomas showing uptake had elevated pSmad2 by IHC (Den Hollander et al. 2015). This was the first study to show therapeutic

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tic antibody delivery to a human brain tumor, reflecting antibody leakage through a damaged blood brain barrier. The metastatic melanoma trial showed some evidence of clinical benefit (even though this was a phase I trial), with six of 29 patients achieving stable disease, including those with mixed tumor responses as assessed by MRI, and one patient with multiple cutaneous lesions showing a 89% partial response that lasted 44 wk (Morris et al. 2014). This was despite the fact that these patients had not responded to other therapies (Morris et al. 2014). Fresolimumab oncology trials were halted for administrative reasons to prioritize efforts on antifibrosis applications. However, with the recent resurgence of interest in TGF- β blockade to enhance immunotherapies; this decision may be reconsidered, especially in light of the fact that some clinical benefit was observed in the first phase I trial for metastatic melanoma.

An 18-patient dose escalation study of LY2382770, a TGF- β 1-specific antiligand IgG4 mAb evaluated by Eli Lilly, dosed once monthly over a range of 20 to 240 mg total antibody per patient per month, was generally found to be safe (the primary endpoint), with fatigue, nausea, and diarrhea as most common side effects (17% of 18 patients) (Cohn et al. 2014). However, most patients discontinued from the protocol after only two cycles of treatment owing to progressive disease with no effect on tumor burden. As designed, the major endpoint of the study was PD analysis of a 37-gene signature of TGF- β expression in circulating mononuclear cells. Despite some marginal pre- to post-treatment changes, the gene expression changes were not consistent or significant, which might account for lack of any clinical response (Cohn et al. 2014). Notably, phase I trials are not designed to test efficacy and lack the statistical power to do so. The apparent lack of concordance between the insignificant clinical outcome data (Cohn et al. 2014) and results using fresolimumab in metastatic melanoma (Morris et al. 2014) might be explained by the need to additionally target TGF- β 2 (and possibly TGF- β 3) to elicit a robust response and/or the insufficient antibody concentration and/or dosing regimen of once per month, because

LY2382770 has a $t_{1/2}$ of only 9 d (Cohn et al. 2014). Metastatic melanoma was not included in the LY2382770 study, so it is possible that discordant results with fresolimumab (Morris et al. 2014) relate to the distinct tumor types investigated between the two studies, with melanoma being particularly sensitive to TGF- β inhibition. A T β RII antibody (IMC-TR1, also known as LY3022859) has also been developed, and the murinized derivative showed an excellent response in mouse models of breast and colon cancer (Zhong et al. 2010). This drug is in phase I trial for patients with advanced solid tumors who have failed standard therapies (NCT01646203).

Small Molecule Kinase Inhibitors

In the field of SMIs, some of the first clinical trials for T β RI inhibitors have recently been published (Fujiwara et al. 2015; Kovacs et al. 2015; Rodón et al. 2015a,b; Sepulveda-Sánchez et al. 2015; Brandes et al. 2016), with more in the pipeline. So far, all published clinical studies used the T β RI SMI drug, galunisertib, whereas an additional 12 interventional trials are ongoing with this drug, and another trial uses the T β RI SMI TEW-7197 (Table 2) (Jin et al. 2014; Son et al. 2014; Naka et al. 2016). Ongoing studies with galunisertib include its use as monotherapy with or without standard of care, such as with the alkylating agents, lomustine or temazolamide in radiochemotherapy for glioblastoma (NCT01682187, NCT01582269, NCT01220271), its use with the antimetabolite, gemcitabine, for metastatic pancreatic cancer (NCT01373164), and with or without sorafenib for hepatocellular carcinoma (NCT01246986, NCT02178358). Other trials include combination or not with radiotherapy for breast (NCT02538471) or rectal cancer (NCT02688712), and with the antiandrogen drug, enzalutamide versus galunisertib versus drug combination in metastatic castration-resistant prostate cancer (NCT02452008). A trial of galunisertib in combination with the checkpoint inhibitor, nivolumab (anti-PD-1, Bristol-Myers Squibb) is recruiting patients with recurrent or advanced NSCLC (NCT02423343).

As discussed earlier, the discovery of serious cardiac toxicity in dogs and rats after continuous administration of any T β RI SMI (Laping et al. 2007; Anderton et al. 2011) necessitates the establishment of a therapeutic window between disease response and cardiotoxicity. These studies were undertaken based on PK/PD modeling in mouse, rat and dog, integrating plasma drug levels and magnitude of inhibition of Smad2 phosphorylation in circulating mononuclear cells, relative to onset of tumor responses versus valvulopathy (Gueorguieva et al. 2014). The therapeutic window turned out to be narrow, between 160 mg and 300 mg per day (administered as two doses of 80–150 mg), with dosing in cycles of 2 weeks on (bi-daily treatment) and 2 weeks off drug.

The FHD study of galunisertib monotherapy with or without lomustine chemotherapy assessed 58 glioblastoma patients, all of whom had relapsed or progressed on prior effective therapies. Most patients were only able to receive two cycles of galunisertib before tumor progression, but clinical benefit was seen in >20% of patients in terms of complete response (CR), partial response (PR), or stable disease (SD) for more than four drug cycles (>5 mo), with a 6.6% complete response rate. Galunisertib monotherapy had better clinical outcomes than combination therapy with lomustine, although this differential is not significant (Rodón et al. 2015b). Intriguingly, the patients who did show a complete or partial response first went through a phase of stable disease, with one patient having a complete response as late as drug cycle 28 (Rodón et al. 2015b). This delayed response was also seen in a smaller study designed to examine clinical responses to a galunisertib–lomustine combination therapy, assessed by dynamic contrast enhanced MRI to quantify vascular brain perfusion and permeability (Sepulveda-Sánchez et al. 2015). In the latter study, two of 12 glioblastoma patients showed clinical benefit, but responses did not begin until the fourth to sixth cycle of galunisertib therapy, contrasting with the normally immediate response (<1 mo) to lomustine chemotherapy. Because one of two responding patients showed reduced cerebral

blood volume and tumor perfusion, as seen in response to the anti-VEGF antibody bevacizumab, the galunisertib effect may have been associated with effects on the vasculature (Sepulveda-Sánchez et al. 2015). However, this late response phenomenon is also a more general feature of cancer immunotherapies (Tuma 2008; Hodi et al. 2010; Hoos et al. 2010), implicating a likely effect of galunisertib on potentiating tumor immunity as well.

A phase II randomized study of 158 recurrent glioblastoma patients treated with galunisertib with or without lomustine compared with lomustine alone, found that the drug combination showed no improvement in OS compared with lomustine plus placebo. However, galunisertib monotherapy was equally effective as lomustine and conferred a clinical advantage in showing less serious hematologic side effects. Tantalizingly, in the FHD study (Rodón et al. 2015b), the tumor drug delivery study (Sepulveda-Sánchez et al. 2015), and the phase II trial (Brandes et al. 2016), galunisertib monotherapy showed a trend toward better outcomes compared with both the lomustine monotherapy and drug combination arms. Not only was this seen in the trend toward increased median OS, but possibly more important, the censoring rate (patient survival at study termination) within the 2-year span of the phase II study was 23% for galunisertib monotherapy versus only 10% for its combination with lomustine, and 15% for lomustine alone (Brandes et al. 2016).

In the phase II study of galunisertib (Brandes et al. 2016), a number of baseline characteristics were measured to screen for prognostic markers. High-baseline levels of circulating CD3⁺ and Foxp3⁺ cells, CD4⁺CD25⁺CD127[−]FOXP3^{LO} cells and eosinophils associated with higher circulating levels of macrophage-derived chemokine (MDC or CCL22). Moreover, high-median MDC was associated with longer OS (11 mo) than low-median MDC (4.7 mo). This correlation was seen across all experimental arms, and has previously been reported as a prognostic factor for glioblastoma in general (Zhou et al. 2015). Circulating TGF- β levels did not significantly associate with OS, al-

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though nine patients with TGF- β 1 levels greater than the median, who were treated with galunisertib monotherapy, had a median OS of 11 mo, compared with an OS of 4.7 mo for the other 30 patients in the same treatment arm whose pretreatment TGF- β 1 levels were less than the median (Brandes et al. 2016). A tentative conclusion from all three trials is that low-grade or secondary gliomas respond better than high-grade tumors, especially those with an *IDH* gene mutation; four out of five (Rodón et al. 2015a,b) and three out of seven (Brandes et al. 2016) *IDH* mutation carriers had CR/PR or SD to galunisertib compared with 12%–17% overall.

In the phase II study of galunisertib, patients with disease progression were offered bevacizumab as an alternative therapy, which may have impacted longer-term outcomes. In fact, there was only one complete response to galunisertib during the 2-year study period (Brandes et al. 2016), despite earlier and smaller studies providing apparently better outcomes. In the FHD study, 16.6% (5/30) and 7.7% (2/26) of glioma patients showed CR or PR, and five patients had SD for more than six cycles of treatment, with two patients surviving for at least 3 years following complete response (Rodón et al. 2015b). Overall, the results of the galunisertib phase II study appear disappointing as they did not reach the endpoint of enhanced efficacy in combination with lomustine. However, the data may be consistent with the possibility that the two drugs antagonized each other. It is, however, encouraging that 235 cancer patients have now been treated with galunisertib, yet show no remarkable cardiovascular toxicities despite, in some cases, treatment over 3 years (Rodón et al. 2015a). Moreover, outcomes in glioblastoma are at least as good as standard of care lomustine, and manifest less severe side effects than the latter. The clinical studies have given credence to a role for galunisertib in depleting glioblastoma stem cells, albeit in only one patient (Anido et al. 2010), and in action on the TME, particularly vascular disruption (Sepulveda-Sánchez et al. 2015). The delayed response to galunisertib therapy may be consistent with changes in tumor cell plasticity, angiogenesis/

vascular stability and/or immune-mediated tumor rejection. The nonadditive nature of the galunisertib and lomustine responses and the suggestive trend toward reduced efficacy of galunisertib when combined with lomustine, might suggest a predominantly immune-mediated mechanism of action, impaired by the alkylating effects of lomustine on immune cells. Galunisertib may indeed be most effective with agents that stimulate rather than deplete the immune system (Garrison et al. 2012; Triplett et al. 2015).

TGF- β BLOCKADE IN COMBINATION WITH CHEMO- OR RADIATION THERAPY

Because TGF- β inhibitors are not directly cytotoxic, it has been postulated that their combined use with cytotoxic chemotherapeutics may be highly efficacious. Several papers have shown the preclinical efficacy of targeting tumors with combinations of cytotoxic drugs and TGF- β blockade. Synergism between the DNA intercalating agent, doxorubicin, and TGF- β inhibition has been shown to suppress breast cancer growth and metastasis in a preclinical model (Bandyopadhyay et al. 2010), and TGF- β inhibitors potentiate the cytotoxic effects of the alkylating agent, melphalan, and the corticosteroid, dexamethasone, in a model of multiple myeloma (Takeuchi et al. 2010). Exposure of multiple myeloma cells to differentiated versus immature MC3T3-E1 pro-osteoblastic cells in cell culture potentiates chemotherapy-induced myeloma cell death. Because TGF- β inhibition promotes osteoblastic differentiation within the bone microenvironment (Yin et al. 1999; Takeuchi et al. 2010), this combinatorial approach holds promise for treatment of other bone tropic metastatic cancers. As mentioned earlier, combining galunisertib with the microtubule disruptor, paclitaxel, gives a better outcome in a mouse model of breast cancer than paclitaxel alone, and the T β R1 SMI, Ki26894, has an additive effect with a fluorouracil analog in reducing tumor growth in a mouse model of scirrhous gastric cell carcinoma (Shinto et al. 2010). However, galunisertib showed no clinical benefit when combined with standard of care

lomustine, an alkylating chemotherapy (Brandes et al. 2016).

There has been considerable interest in combining TGF- β pathway inhibition and radiotherapy (Anscher et al. 2008; Bouquet et al. 2011; Zhang et al. 2011). Radiation physically activates the latent TGF- β complex in cell culture and in vivo (Barcellos-Hoff 1993; Barcellos-Hoff et al. 1994). In cells in culture, radiation induces biological release of TGF- β as part of the radiation-induced stress response. TGF- β plays an important role in supporting the DNA damage repair pathway, particularly through activation of p53 and phosphorylation of ataxia telangiectasia mutated (ATM), a serine/threonine kinase that is recruited and activated by DNA double-strand breaks. LY2109761, which targets the kinase activities of T β RII and T β RI (Sawyer et al. 2003; Yingling et al. 2004), and the antipan TGF- β antibody, 1D11 (Morris et al. 2014), attenuate radiation-induced activation of p53 and ATM in breast cancer cells in culture and in vivo, suggesting that TGF- β signaling may potentiate therapeutic killing of tumors by preventing DNA repair and accentuating the cytotoxic effect of radiation (Bouquet et al. 2011). Furthermore, TGF- β blockade with the 1D11 antibody significantly stimulates the anticancer immune response against implanted mammary tumors (4T1 cells) following RT, resulting in tumor clearance when combined with RT (Vanpouille-Box et al. 2015). Even short-term dosing with TGF- β inhibitors might provide a considerable therapeutic window in potentiating radiotherapy. Moreover, RT can cause disfiguring and painful fibrosis, itself a target for anti-TGF- β therapy, possibly providing an added benefit of TGF- β inhibition (Anscher et al. 2008). Thus, TGF- β blockade may have a triad of benefits, stimulating radiation-induced tumor cell killing (Bouquet et al. 2011), augmenting immune rejection (Vanpouille-Box et al. 2015), while limiting radiation-induced fibrosis (Rabbani et al. 2003; Anscher et al. 2008). This approach is part of an active clinical trial of fresolimumab in combination with radiotherapy for metastatic breast cancer (NCT01401062).

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TGF- β SIGNALING BLOCKADE IN IMMUNOTHERAPY

Although most clinical trials of TGF- β blockade have not shown dramatic clinical benefit by classical RECIST (response evaluation criteria in solid tumors) (Therasse et al. 2000), it is still early days in TGF- β blockade trials, with only some drugs tested so far in phase I dose escalation studies for assessment of safety. Placing the first phase I and II clinical data into context, one has to consider that even a blockbuster immunotherapy agent such as ipilimumab (anti-CTLA-4) showed similar deficiencies in its first clinical trials. An early clinical trial of ipilimumab in metastatic melanoma showed an objective clinical response in only five out of 46 patients (11%), at the expense of 35% of patients having grade three or four autoimmune toxicities (Maker et al. 2006), which is not so different from the metastatic melanoma response to fresolimumab (six of 29 patients achieving stable disease, one for ≥ 44 wk) (Morris et al. 2014). It is now generally accepted that the classical RECIST or World Health Organization response criteria developed for patients on cytotoxic drugs are not appropriate for assessing immunotherapies (Tuma 2008; Hoos et al. 2010). For example, responses to checkpoint inhibitors such as ipilimumab or nivolumab are frequently considerably delayed, with a protracted period of months of stable disease or even tumor progression before antitumor activity becomes apparent. Comparison of median OS may not be an adequate parameter when only a small fraction of patients shows a response, even though the response may be complete, robust, and durable, possibly lasting for years. A more significant measure should therefore be assessment of the fraction of patients with long-term durable responses, which was not an endpoint in the TGF- β blockade trials. The fraction of glioblastoma or metastatic melanoma patients achieving long-term durable responses after galunisertib or fresolimumab therapy (Morris et al. 2014; Brandes et al. 2016), may become significant if combined with other drugs. Bearing in mind the safety of TGF- β signaling inhibitors at the doses used, the outlook for use of

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these drugs as monotherapy or as adjunct to other immunotherapy agents is very good.

Proof-of-principle usage of TGF- β blockade for enhancement of immunotherapy has been shown preclinically using LY2109761 in adoptive T-cell therapy (Wallace et al. 2008), and using the normally unstable T β RI SMI, SB505124, encapsulated in a slow-release biodegradable polymeric nanoparticle for the stimulation of interleukin 2 (IL-2) therapy of melanoma (Park et al. 2012). A novel approach has been taken by generating mRNA encoding a “fusokine,” that is, a fusion protein of the immune stimulant, interferon- β , with the ectodomain of T β RII, which acts as a TGF- β “sink.” DCs exposed to this fusokine take up the RNA by phagocytosis and express the encoded fusion protein, resulting in enhanced antigen-presenting capacity. The fusokine also attenuates the suppressor activity of myeloid-derived suppressor cells (MDSCs) after transfection of the encoding plasmid into these cells (Van Der Jeught et al. 2014). It has been postulated that intratumoral delivery of this fusokine RNA might enhance antigen presentation, and thus tumor immunity after intratumoral injection in vivo, but this remains to be tested. Finally, an agonist antibody against the immune activating cell surface receptor, Ox40, which is expressed on CD4⁺ and CD8⁺ cells, has been shown to synergize with the T β RI SMI, SM16, leading to complete tumor regression in models of colon cancer (CT26) and chemically induced sarcoma (MCA205) (Triplett et al. 2015).

FUTURE DIRECTIONS WITH TGF- β BLOCKADE

Several clinical oncology trials with galunisertib, fresolimumab, and LY3022859 are listed as ongoing by ClinicalTrials.gov (Table 2), and the initial reports suggest that galunisertib is at least as efficacious as lomustine for glioblastoma and may have fewer side effects. Nevertheless, progress through the clinical pipeline has been slow. It may be that the window between antitumor efficacy and toxicological effects has been considered too narrow for general use, especially considering that genetic variation between in-

dividuals helps define the outcomes of germline genetic loss of TGF- β signaling components, and thus might influence both therapeutic drug responses and on-target side effects (Bonyadi et al. 1997; Akhurst 2004; Benzinou et al. 2012; Chung Kang et al. 2013; Kawasaki et al. 2014). Taking into account the many activities of TGF- β and its pleiotropic actions, major efforts focus on identifying alternative targets that influence the TGF- β signaling pathway, with a strong incentive to identify drugs that might block TGF- β 's tumor progressing activities while sparing tumor-suppressing activities. Many studies identified pivotal intratumoral players that regulate such a balance—for example, disabled homolog 2 (DAB2), which is epigenetically down-regulated in human SCCs, resulting in attenuation of TGF- β tumor suppressor activity to enable TGF- β tumor-promoting activities, including promotion of cell motility, anchorage-independent growth, and in vivo tumor growth (Hannigan et al. 2010). Another example includes the pivotal role played by differential expression of three isoforms of C/EBP β that are generated by usage of distinct translational start sites during tumor progression. In normal cells, the major C/EBP β isoforms LAP1 and LAP2 bind pSmads to elicit a growth suppressive transcriptional response. However, as tumors progress, there is preferential translation of a third truncated isoform, LIP, which lacks the amino-terminal domain and acts in a dominant-negative manner to suppress the growth inhibitory action of LAP1/2 during cancer progression (Gomis et al. 2006b). Targeting such intracellular players is however likely destined for failure because of the incredible plasticity and evolution of tumor cells, which evolve rapidly to circumnavigate signaling pathway blockade, as exemplified by clinical trials with drugs that target other pathways, such as HER2 (Johannessen et al. 2010; Nazarian et al. 2010) and B-Raf (Sergina et al. 2007). A more fruitful approach may be to target proteins with a more restricted tissue expression profile than that of the TGF- β receptors, which might affect the TGF- β activity only in specific cells of the TME such as in DCs, NK cells, MDSCs and/or T cells. Integrins that activate TGF- β have been

considered as such drug targets (Van Aarsen et al. 2008; Dutta et al. 2014). Another example is GARP, which is required for activation of latent TGF- β on Tregs and platelets (Stockis et al. 2009; Tran et al. 2009). Blocking the interaction of GARP with latent TGF- β at the surface of Treg cells using antibodies that bind a specific conformational motif of GARP could block TGF- β activation, and, consequently, also the immunosuppressive activity of Tregs (Cuende et al. 2015). This antibody may therefore impose a more specific TGF- β blockade immunotherapy for cancer, probably with fewer side effects than blocking TGF- β receptors or ligands, because of the restricted pattern of GARP expression. Other TGF- β modulatory drugs are in development for various disease applications including oncology (Van Aarsen et al. 2008; Henderson et al. 2013; Salvo et al. 2014).

CONCLUDING REMARKS

Compared with many other anticancer drugs in use, TGF- β blockade would, so far, appear relatively safe. For example, ipilimumab (anti-CTLA4) has shown major successes as an immune checkpoint blockade agent for melanoma but has considerable toxicity in some patients, causing GI inflammation, asthma, vitiligo, and other autoimmune-like diseases (Spain et al. 2016). Despite this major caveat, the drug is still in considerable demand by patients, but requires close monitoring for drug-related autoimmune toxicities. The next stage in drug development for TGF- β blockade will be discovering the most efficacious drug combinations for each oncological disease application, whether this is radiation, chemotherapy, pathway targeted therapies and/or immunotherapy, and defining the most potent dosing regimens. It is possible that antiligand or antireceptor drugs may not provide a large enough therapeutic window between therapeutic and toxicological responses. This could be remediated by optimized dosing strategies, but may require the development of new drugs against tissue-specific TGF- β signaling components that are active only on discrete cell types of the TME, such as GARP. Finally, if TGF- β blockade drugs are

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approved for widespread use, there will be considerable variation in both therapeutic and toxicological responses among patients (Bonyadi et al. 1997; Akhurst 2004; Valle et al. 2008; Benzinou et al. 2012; Chung Kang et al. 2013; Kawasaki et al. 2014). There is therefore a growing need for development of predictive biomarkers of TGF- β blockade (Gueorguieva et al. 2014; Kawasaki et al. 2014).

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REFERENCES

- Akhurst RJ. 2004. TGF- β signaling in health and disease. *Nat Genet* **36**: 790–792.
- Akhurst RJ, Derynck R. 2001. TGF- β signaling in cancer—A double-edged sword. *Trends Cell Biol* **11**: S44–51.
- Akhurst RJ, Hata A. 2012. Targeting the TGF- β signalling pathway in disease. *Nat Rev Drug Discov* **11**: 790–811.
- Akhurst RJ, Fee F, Balmain A. 1988. Localized production of TGF- β mRNA in tumour promoter-stimulated mouse epidermis. *Nature* **331**: 363–365.
- Anderton MJ, Mellor HR, Bell A, Sadler C, Pass M, Powell S, Steele SJ, Roberts RR, Heier A. 2011. Induction of heart valve lesions by small-molecule ALK5 inhibitors. *Toxicol Pathol* **39**: 916–924.
- Anido J, Sáez-Borderías A, González-Juncà A, Rodón L, Folch G, Carmona MA, Prieto-Sánchez RM, Barba I, Martínez-Sáez E, Prudkin L, et al. 2010. TGF- β receptor inhibitors target the CD44^{high}/Id1^{high} glioma-initiating cell population in human glioblastoma. *Cancer Cell* **18**: 655–668.
- Annes JP, Munger JS, Rifkin DB. 2003. Making sense of latent TGF- β activation. *J Cell Sci* **116**: 217–224.
- Annes JP, Chen Y, Munger JS, Rifkin DB. 2004. Integrin α V β 6-mediated activation of latent TGF- β requires the latent TGF- β binding protein-1. *J Cell Biol* **165**: 723–734.
- Anscher MS, Thrasher B, Zgonjanin L, Rabbani ZN, Corbley MJ, Fu K, Sun L, Lee WC, Ling LE, Vujaskovic Z. 2008. Small molecular inhibitor of transforming growth factor- β protects against development of radiation-induced lung injury. *Int J Radiat Oncol Biol Phys* **71**: 829–837.
- Arnault JB, Mateus C, Escudier B, Tomasic G, Wechsler J, Hollville E, Soria JC, Malka D, Sarasin A, Larcher M, et al. 2012. Skin tumors induced by sorafenib; paradoxical RAS-

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- RAF pathway activation and oncogenic mutations of HRAS, TP53, and TGFBR1. *Clin Cancer Res* **18**: 263–272.
- Arnold T, Niaudet C, Pang MF, Siegenthaler J, Gaengel K, Jung B, Ferrero GM, Mukoyama YS, Fuxe J, Akhurst R, et al. 2014. Excessive vascular sprouting underlies cerebral hemorrhage in mice lacking integrin α V β 8-TGF- β signaling in the brain. *Development* **141**: 4489–4499.
- Arteaga CL, Hurd SD, Winnier AR, Johnson MD, Fendly BM, Forbes JT. 1993. Anti-transforming growth factor (TGF)- β antibodies inhibit breast cancer cell tumorigenicity and increase mouse spleen natural killer cell activity. Implications for a possible role of tumor cell/host TGF- β interactions in human breast cancer progression. *J Clin Invest* **92**: 2569–2576.
- Ask K, Bonniaud P, Maass K, Eickelberg O, Margetts PJ, Warburton D, Groffen J, Gaudie J, Kolb M. 2008. Progressive pulmonary fibrosis is mediated by TGF- β isoform 1 but not TGF- β 3. *Int J Biochem Cell Biol* **40**: 484–495.
- Bandyopadhyay A, Wang L, Agyin J, Tang Y, Lin S, Yeh IT, De K, Sun LZ. 2010. Doxorubicin in combination with a small TGF- β inhibitor: A potential novel therapy for metastatic breast cancer in mouse models. *PLoS ONE* **5**: e10365.
- Barcellos-Hoff MH. 1993. Radiation-induced transforming growth factor β and subsequent extracellular matrix reorganization in murine mammary gland. *Cancer Res* **53**: 3880–3886.
- Barcellos-Hoff MH, Dix TA. 1996. Redox-mediated activation of latent transforming growth factor- β 1. *Mol Endocrinol* **10**: 1077–1083.
- Barcellos-Hoff MH, Derynck R, Tsang ML, Weatherbee JA. 1994. Transforming growth factor- β activation in irradiated murine mammary gland. *J Clin Invest* **93**: 892–899.
- Bedinger D, Lao L, Khan S, Lee S, Takeuchi T, Mirza AM. 2016. Development and characterization of human monoclonal antibodies that neutralize multiple TGF β isoforms. *mAbs* **8**: 389–404.
- Bellone G, Turletti A, Artusio E, Mareschi K, Carbone A, Tibaudi D, Robecchi A, Emanuelli G, Rodeck U. 1999. Tumor-associated transforming growth factor- β and interleukin-10 contribute to a systemic Th2 immune phenotype in pancreatic carcinoma patients. *Am J Pathol* **155**: 537–547.
- Bellone G, Carbone A, Tibaudi D, Mauri F, Ferrero I, Smirne C, Suman F, Rivetti C, Migliaretti G, Camandona M, et al. 2001. Differential expression of transforming growth factors- β 1, - β 2 and - β 3 in human colon carcinoma. *Eur J Cancer* **37**: 224–233.
- Benvenuto GM, Ometto R, Fontanelli A, Fortunato A, Ruffini PA, Fossier V, Morandi P. 2003. Chemotherapy-related cardiotoxicity: New diagnostic and preventive strategies. *Italian Heart J* **4**: 655–667.
- Benzinou M, Clermont FF, Letteboer TG, Kim JH, Espejel S, Harradine KA, Arbelaez J, Luu MT, Roy R, Quigley D, et al. 2012. Mouse and human strategies identify PTPN14 as a modifier of angiogenesis and hereditary haemorrhagic telangiectasia. *Nat Commun* **3**: 616.
- Bhola NE, Balko JM, Dugger TC, Kuba MG, Sánchez V, Sanders M, Stanford J, Cook RS, Arteaga CL. 2013. TGF- β inhibition enhances chemotherapy action against triple-negative breast cancer. *J Clin Invest* **123**: 1348–1358.
- Bogdahn U, Hau P, Stockhammer G, Venkataramana NK, Mahapatra AK, Suri A, Balasubramaniam A, Nair S, Oliushine V, Parfenov V, et al. 2011. Targeted therapy for high-grade glioma with the TGF- β 2 inhibitor trabedersen: Results of a randomized and controlled phase IIb study. *Neuro Oncol* **13**: 132–142.
- Bonyadi M, Rusholme SA, Cousins FM, Su HC, Biron CA, Farrall M, Akhurst RJ. 1997. Mapping of a major genetic modifier of embryonic lethality in TGF- β 1 knockout mice. *Nat Genet* **15**: 207–211.
- Bouquet F, Pal A, Pilonis KA, Demaria S, Hann B, Akhurst RJ, Babb JS, Lonning SM, DeWyngaert JK, Formenti SC, et al. 2011. TGF- β 1 inhibition increases the radiosensitivity of breast cancer cells in vitro and promotes tumor control by radiation in vivo. *Clin Cancer Res* **17**: 6754–6765.
- Bragado P, Estrada Y, Parikh F, Krause S, Capobianco C, Farina HG, Schewe DM, Aguirre-Ghiso JA. 2013. TGF- β 2 dictates disseminated tumour cell fate in target organs through TGF- β –RIII and p38 α / β signalling. *Nat Cell Biol* **15**: 1351–1361.
- Brandes AA, Carpentier AF, Kesari S, Sepulveda-Sánchez JM, Wheeler HR, Chinot O, Cher L, Steinbach JP, Capper D, Specenier P, et al. 2016. A phase II randomized study of galunisertib monotherapy or galunisertib plus lomustine compared with lomustine monotherapy in patients with recurrent glioblastoma. *Neuro Oncol* **18**: 1146–1156.
- Brown JD, DiChiara MR, Anderson KR, Gimbrone MA Jr, Topper JN. 1999. MEKK-1, a component of the stress (stress-activated protein kinase/c-Jun N-terminal kinase) pathway, can selectively activate Smad2-mediated transcriptional activation in endothelial cells. *J Biol Chem* **274**: 8797–8805.
- Bueno L, De Alwis DP, Pitou C, Yingling J, Lahn M, Glatt S, Troconiz IF. 2008. Semi-mechanistic modelling of the tumour growth inhibitory effects of LY2157299, a new type I receptor TGF- β kinase antagonist, in mice. *Eur J Cancer* **44**: 142–150.
- Caulin C, Scholl FG, Frontelo P, Gamallo C, Quintanilla M. 1995. Chronic exposure of cultured transformed mouse epidermal cells to transforming growth factor- β 1 induces an epithelial-mesenchymal transdifferentiation and a spindle tumoral phenotype. *Cell Growth Diff* **6**: 1027–1035.
- Chamberlain MC. 2011. Convection-enhanced delivery of a transforming growth factor- β 2 inhibitor trabedersen for recurrent high-grade gliomas: Efficacy real or imagined? *Neuro Oncol* **13**: 558–559.
- Chaudhry SS, Cain SA, Morgan A, Dallas SL, Shuttleworth CA, Kielty CM. 2007. Fibrillin-1 regulates the bioavailability of TGF- β 1. *J Cell Biol* **176**: 355–367.
- Chen ML, Pittet MJ, Gorelik L, Flavell RA, Weissleder R, Von Boehmer H, Khazaie K. 2005. Regulatory T cells suppress tumor-specific CD8 T cell cytotoxicity through TGF- β signals in vivo. *Proc Natl Acad Sci* **102**: 419–424.
- Chen M, Matsuda H, Wang L, Watanabe T, Kimura MT, Igarashi J, Wang X, Sakimoto T, Fukuda N, Sawa M, et al. 2010. Pretranscriptional regulation of TGF- β 1 by PI polyamide prevents scarring and accelerates wound heal-

- ing of the cornea after exposure to alkali. *Mol Therapy* **18**: 519–527.
- Chung Kang H, Quigley DA, Kim IJ, Wakabayashi Y, Ferguson-Smith MA, D'Alessandro M, Birgitte Lane E, Akhurst RJ, Goudie DR, Balmain A. 2013. Multiple self-healing squamous epithelioma (MSSE): Rare variants in an adjacent region of chromosome 9q22.3 to known *TGFBR1* mutations suggest a digenic or multi-locus etiology. *J Invest Derm* **133**: 1907–1910.
- Coffey RJ Jr, Sipes NJ, Bascom CC, Graves-Deal R, Pennington CY, Weissman BE, Moses HL. 1988. Growth modulation of mouse keratinocytes by transforming growth factors. *Cancer Res* **48**: 1596–1602.
- Cohn A, Lahn MM, Williams KE, Cleverly AL, Pitou C, Kadam SK, Farman MW, Desai D, Raju R, Conkling P, et al. 2014. A phase I dose-escalation study to a pre-defined dose of a transforming growth factor- β 1 monoclonal antibody (T β M1) in patients with metastatic cancer. *Int J Oncol* **45**: 2221–2231.
- Connolly EC, Saunier EF, Quigley D, Luu MT, De Sapio A, Hann B, Yingling JM, Akhurst RJ. 2011. Outgrowth of drug-resistant carcinomas expressing markers of tumor aggression after long term T β R1/II kinase inhibition with LY2109761. *Cancer Res* **71**: 1–11.
- Constam DB, Philipp J, Malipiero UV, ten Dijke P, Schachner M, Fontana A. 1992. Differential expression of transforming growth factor- β 1, - β 2, and - β 3 by glioblastoma cells, astrocytes, and microglia. *J Immunol* **148**: 1404–1410.
- Corcoran RB, Dias-Santagata D, Bergeth K, Lafrate AJ, Settleman J, Engelman JA. 2010. *BRAF* gene amplification can promote acquired resistance to MEK inhibitors in cancer cells harboring the *BRAF* V600E mutation. *Sci Signal* **3**: ra84.
- Cuende J, Liénart S, Dedobbeleer O, van der Woning B, De Boeck G, Stockis J, Huygens C, Colau D, Somja J, Delvenne P, et al. 2015. Monoclonal antibodies against GARP/TGF- β 1 complexes inhibit the immunosuppressive activity of human regulatory T cells in vivo. *Sci Transl Med* **7**: 284ra256.
- Cui W, Fowles DJ, Cousins FM, Duffie E, Bryson S, Balmain A, Akhurst RJ. 1995. Concerted action of TGF- β 1 and its type II receptor in control of epidermal homeostasis in transgenic mice. *Genes Dev* **9**: 945–955.
- Dang CV, O'Donnell KA, Juopperi T. 2005. The great MYC escape in tumorigenesis. *Cancer Cell* **8**: 177–178.
- Davies M, Robinson M, Smith E, Huntley S, Prime S, Paterson I. 2005. Induction of an epithelial to mesenchymal transition in human immortal and malignant keratinocytes by TGF- β 1 involves MAPK, Smad and AP-1 signaling pathways. *J Cell Biochem* **95**: 918–931.
- den Hollander MW, Bensch F, Glaudemans AW, Oude Munink TH, Enting RH, den Dunnen WF, Heesters MA, Kruij FA, Lub-de Hooge MN, Cees de Groot J, et al. 2015. TGF- β antibody uptake in recurrent high-grade glioma imaged with ^{89}Zr -Fresolimumab PET. *J Nuc Med* **56**: 1310–1314.
- Derynck R, Akhurst RJ. 2007. Differentiation plasticity regulated by TGF- β family proteins in development and disease. *Nat Cell Biol* **9**: 1000–1004.
- Derynck R, Zhang YE. 2003. Smad-dependent and Smad-independent pathways in TGF- β family signalling. *Nature* **425**: 577–584.
- Derynck R, Goeddel DV, Ullrich A, Gutterman JU, Williams RD, Bringman TS, Berger WH. 1987. Synthesis of messenger RNAs for transforming growth factors α and β and the epidermal growth factor receptor by human tumors. *Cancer Res* **47**: 707–712.
- Derynck R, Akhurst RJ, Balmain A. 2001. TGF- β signaling in tumor suppression and cancer progression. *Nat Genet* **29**: 117–129.
- Descargues P, Sil AK, Sano Y, Korchynskyi O, Han G, Owens P, Wang XJ, Karin M. 2008. IKK α is a critical coregulator of a Smad4-independent TGF β -Smad2/3 signaling pathway that controls keratinocyte differentiation. *Proc Natl Acad Sci* **105**: 2487–92.
- Dickson MC, Martin JS, Cousins FM, Kulkarni AB, Karlsson S, Akhurst RJ. 1995. Defective haematopoiesis and vasculogenesis in transforming growth factor- β 1 knock out mice. *Development* **121**: 1845–1854.
- Dong Z, Guo W, Guo Y, Kuang G, Yang Z. 2012. Concordant promoter methylation of transforming growth factor- β receptor types I and II occurs early in esophageal squamous cell carcinoma. *Am J Med Sci* **343**: 375–381.
- Dong X, Hudson NE, Lu C, Springer TA. 2014. Structural determinants of integrin β -subunit specificity for latent TGF- β . *Nat Struct Mol Biol* **21**: 1091–1096.
- Dutta A, Li J, Lu H, Akech J, Pratap J, Wang T, Zerlanko BJ, FitzGerald TJ, Jiang Z, Birbe R, et al. 2014. Integrin α v β 6 promotes an osteolytic program in cancer cells by up-regulating MMP2. *Cancer Res* **74**: 1598–1608.
- Ehata S, Johansson E, Katayama R, Koike S, Watanabe A, Hoshino Y, Katsuno Y, Komuro A, Koinuma D, Kano MR, et al. 2011. Transforming growth factor- β decreases the cancer-initiating cell population within diffuse-type gastric carcinoma cells. *Oncogene* **30**: 1693–1705.
- Engel ME, McDonnell MA, Law BK, Moses HL. 1999. Interdependent SMAD and JNK signaling in transforming growth factor- β -mediated transcription. *J Biol Chem* **274**: 37413–37420.
- Engelman JA, Settleman J. 2008. Acquired resistance to tyrosine kinase inhibitors during cancer therapy. *Curr Opin Genet Dev* **18**: 73–79.
- Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, Lindeman N, Gale CM, Zhao X, Christensen J, et al. 2007. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science* **316**: 1039–1043.
- Flanders KC, Wakefield LM. 2009. Transforming growth factor- β s and mammary gland involution: Functional roles and implications for cancer progression. *J Mammary Gland Biol Neoplasia* **14**: 131–144.
- Flavell RA, Sanjabi S, Wrzesinski SH, Licona-Limon P. 2010. The polarization of immune cells in the tumour environment by TGF- β . *Nat Rev Immunol* **10**: 554–567.
- Fowles DJ, Flanders KC, Duffie E, Balmain A, Akhurst RJ. 1992. Discordant transforming growth factor- β 1 RNA and protein localization during chemical carcinogenesis of the skin. *Cell Growth Diff* **3**: 81–91.
- Fransvea E, Angelotti U, Antonaci S, Giannelli G. 2008. Blocking transforming growth factor- β up-regulates E-

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- cadherin and reduces migration and invasion of hepatocellular carcinoma cells. *Hepatology* **47**: 1557–1566.
- Frazier K, Thomas R, Scicchitano M, Mirabile R, Boyce R, Zimmerman D, Grygielko E, Nold J, DeGouville AC, Huet S, et al. 2007. Inhibition of ALK5 signaling induces physeal dysplasia in rats. *Toxicol Pathol* **35**: 284–295.
- Fridlender ZG, Sun J, Kim S, Kapoor V, Cheng G, Ling L, Worthen GS, Albelda SM. 2009. Polarization of tumor-associated neutrophil phenotype by TGF- β : “N1” versus “N2” TAN. *Cancer Cell* **16**: 183–194.
- Fujii M, Nakanishi H, Toyoda T, Tanaka I, Kondo Y, Osada H, Sekido Y. 2012a. Convergent signaling in the regulation of connective tissue growth factor in malignant mesothelioma: TGF- β signaling and defects in the Hippo signaling cascade. *Cell Cycle* **11**: 3373–3379.
- Fujii M, Toyoda T, Nakanishi H, Yatabe Y, Sato A, Matsudaira Y, Ito H, Murakami H, Kondo Y, Kondo E, et al. 2012b. TGF- β synergizes with defects in the Hippo pathway to stimulate human malignant mesothelioma growth. *J Exp Med* **209**: 479–494.
- Fujiwara Y, Nokihara H, Yamada Y, Yamamoto N, Sunami K, Utsumi H, Asou H, Takahashi O, Ogasawara K, Gueorguieva I, et al. 2015. Phase I study of galunisertib, a TGF- β receptor I kinase inhibitor, in Japanese patients with advanced solid tumors. *Cancer Chemo Pharm* **76**: 1143–1152.
- Garrison K, Hahn T, Lee WC, Ling LE, Weinberg AD, Akporiaye ET. 2012. The small molecule TGF- β signaling inhibitor SM16 synergizes with agonistic OX40 antibody to suppress established mammary tumors and reduce spontaneous metastasis. *Cancer Immunol Immunother* **61**: 511–521.
- Giaccone G, Bazhenova LA, Nemunaitis J, Tan M, Juhász E, Ramlau R, van den Heuvel MM, Lal R, Kloecker GH, Eaton KD, et al. 2015. A phase III study of belagenpumatucel-L, an allogeneic tumour cell vaccine, as maintenance therapy for non-small cell lung cancer. *Eur J Cancer Res* **51**: 2321–2329.
- Giampieri S, Pinner S, Sahai E. 2010. Intravital imaging illuminates transforming growth factor β signaling switches during metastasis. *Cancer Res* **70**: 3435–3439.
- Giehl K, Imamichi Y, Menke A. 2007. Smad4-independent TGF- β signaling in tumor cell migration. *Cells Tissues Organs* **185**: 123–130.
- Gill S, June CH. 2015. Going viral: Chimeric antigen receptor T-cell therapy for hematological malignancies. *Immunol Rev* **263**: 68–89.
- Gomis RR, Alarcon C, He W, Wang Q, Seoane J, Lash A, Massagué J. 2006a. A FoxO-Smad synexpression group in human keratinocytes. *Proc Natl Acad Sci* **103**: 12747–12752.
- Gomis RR, Alarcon C, Nadal C, Van Poznak C, Massagué J. 2006b. C/EBP β at the core of the TGF β cytostatic response and its evasion in metastatic breast cancer cells. *Cancer Cell* **10**: 203–214.
- Gorelik L, Flavell RA. 2001. Immune-mediated eradication of tumors through the blockade of transforming growth factor- β signaling in T cells. *Nat Med* **7**: 1118–1122.
- Gorelik L, Flavell RA. 2002. Transforming growth factor- β in T-cell biology. *Nat Rev Immunol* **2**: 46–53.
- Gorsch SM, Memoli VA, Stukel TA, Gold LI, Arrick BA. 1992. Immunohistochemical staining for transforming growth factor β 1 associates with disease progression in human breast cancer. *Cancer Res* **52**: 6949–6952.
- Gridley DS, Sura SS, Uhm JR, Lin CH, Kettering JD. 1993. Effects of anti-transforming growth factor- β antibody and interleukin-2 in tumor-bearing mice. *Cancer Biother* **8**: 159–170.
- Grönroos E, Kingston IJ, Ramachandran A, Randall RA, Vizan P, Hill CS. 2012. Transforming growth factor β inhibits bone morphogenetic protein-induced transcription through novel phosphorylated Smad1/5-Smad3 complexes. *Mol Cell Biol* **32**: 2904–2916.
- Gudey SK, Sundar R, Mu Y, Wallenius A, Zang G, Bergh A, Heldin CH, Landström M. 2014. TRAF6 stimulates the tumor-promoting effects of TGF β type I receptor through polyubiquitination and activation of presenilin 1. *Sci Signal* **7**: ra2.
- Gueorguieva I, Cleverly AL, Stauber A, Sada Pillay N, Rodon JA, Miles CP, Yingling JM, Lahn MM. 2014. Defining a therapeutic window for the novel TGF- β inhibitor LY2157299 monohydrate based on a pharmacokinetic/pharmacodynamic model. *Br J Clin Pharmacol* **77**: 796–807.
- Hannigan A, Smith P, Kalna G, Lo Nigro C, Orange C, O’Brien DI, Shah R, Syed N, Spender LC, Herrera B, et al. 2010. Epigenetic downregulation of human disabled homolog 2 switches TGF- β from a tumor suppressor to a tumor promoter. *J Clin Invest* **120**: 2842–2857.
- Harradine KA, Ridd K, Saunier EF, Clermont FF, Perez-Lozada J, Moore DH, Epstein EH Jr, Bastian BC, Akhurst RJ. 2009. Elevated cutaneous Smad activation associates with enhanced skin tumor susceptibility in organ transplant recipients. *Clin Cancer Res* **15**: 5101–5107.
- Hatsell SJ, Idone V, Wolken DM, Huang L, Kim HJ, Wang L, Wen X, Nannuru KC, Jimenez J, Xie L, et al. 2015. ACVR1R206H receptor mutation causes fibrodysplasia ossificans progressiva by imparting responsiveness to activin A. *Sci Transl Med* **7**: 303ra137.
- Hau P, Jachimczak P, Bogdahn U. 2009. Treatment of malignant gliomas with TGF- β 2 antisense oligonucleotides. *Expert Rev Anticancer Ther* **9**: 1663–1674.
- Heldin CH, Rubin K, Pietras K, Östman A. 2004. High interstitial fluid pressure—An obstacle in cancer therapy. *Nat Rev Cancer* **4**: 806–813.
- Heldin CH, Landström M, Moustakas A. 2009. Mechanism of TGF- β signaling to growth arrest, apoptosis, and epithelial–mesenchymal transition. *Curr Opin Cell Biol* **21**: 166–176.
- Henderson NC, Arnold TD, Katamura Y, Giacomini MM, Rodriguez JD, McCarty JH, Pellicoro A, Raschperger E, Betsholtz C, Ruminski PG, et al. 2013. Targeting of α_v integrin identifies a core molecular pathway that regulates fibrosis in several organs. *Nat Med* **19**: 1617–1624.
- Hocevar BA, Brown TL, Howe PH. 1999. TGF- β induces fibronectin synthesis through a c-Jun N-terminal kinase-dependent, Smad4-independent pathway. *EMBO J* **18**: 1345–1356.
- Hocevar BA, Prunier C, Howe PH. 2005. Disabled-2 (Dab2) mediates transforming growth factor β (TGF- β)-stimulated fibronectin synthesis through TGF- β -activated ki-

- nase 1 and activation of the JNK pathway. *J Biol Chem* **280**: 25920–25927.
- Hodi FS, O'Day SJ, McDermott DE, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, et al. 2010. Improved survival with ipilimumab in patients with metastatic melanoma. *N Eng J Med* **363**: 711–723.
- Hoos A, Eggermont AM, Janetzki S, Hodi FS, Ibrahim R, Anderson A, Humphrey R, Blumenstein B, Old L, Wolchok J. 2010. Improved endpoints for cancer immunotherapy trials. *J Natl Can Inst* **102**: 1388–1397.
- Hoot KE, Lighthall J, Han G, Lu SL, Li A, Ju W, Kulesz-Martin M, Bottinger E, Wang XJ. 2008. Keratinocyte-specific Smad2 ablation results in increased epithelial-mesenchymal transition during skin cancer formation and progression. *J Clin Invest* **118**: 2722–2732.
- Huang S, Hölzel M, Knijnenburg T, Schlicker A, Roepman P, McDermott U, Garnett M, Grønrum W, Sun C, Prahalad A, et al. 2012. MED12 controls the response to multiple cancer drugs through regulation of TGF- β receptor signaling. *Cell* **151**: 937–950.
- Iacobuzio-Donahue CA, Fu B, Yachida S, Luo M, Abe H, Henderson CM, Villardell F, Wang Z, Keller JW, Banerjee P, et al. 2009. DPC4 gene status of the primary carcinoma correlates with patterns of failure in patients with pancreatic cancer. *J Clin Oncol* **27**: 1806–1813.
- Igarashi J, Fukuda N, Inoue T, Nakai S, Saito K, Fujiwara K, Matsuda H, Ueno T, Matsumoto Y, Watanabe T, et al. 2015. Preclinical study of novel gene silencer pyrrole-imidazole polyamide targeting human TGF- β 1 promoter for hypertrophic scars in a common marmoset primate model. *PLoS ONE* **10**: e0125295.
- James D, Nam HS, Seandel M, Nolan D, Janovitz T, Tomishima M, Studer L, Lee G, Lyden D, Benezra R, et al. 2010. Expansion and maintenance of human embryonic stem cell-derived endothelial cells by TGF β inhibition is Id1-dependent. *Nat Biotech* **28**: 161–166.
- Jia ZC, Zou LY, Ni B, Wan Y, Zhou W, Lv YB, Geng M, Wu YZ. 2005. Effective induction of antitumor immunity by immunization with plasmid DNA encoding TRP-2 plus neutralization of TGF- β . *Cancer Immunol Immunother* **54**: 446–452.
- Jin CH, Krishnaiah M, Sreenu D. 2014. Discovery of *N*-((4-([1,2,4]triazolo[1,5-*a*]pyridin-6-yl)-5-(6-methylpyridin-2-yl)-1*H*-imidazol-2-yl)methyl)-2-fluoroaniline (EW-7197): A highly potent, selective, and orally bioavailable inhibitor of TGF- β type I receptor kinase as cancer immunotherapeutic/antifibrotic agent. *J Med Chem* **57**: 4213–4238.
- Jobling MF, Mott JD, Finnegan MT, Jurukovski V, Erickson AC, Walian PJ, Taylor SE, Ledbetter S, Lawrence CM, Rifkin DB, et al. 2006. Isoform-specific activation of latent transforming growth factor β (LTGF- β) by reactive oxygen species. *Radiat Res* **166**: 839–848.
- Johannessen CM, Boehm JS, Kim SY, Thomas SR, Wardwell L, Johnson LA, Emery CM, Stransky N, Cogdill AP, Barretina J, et al. 2010. COT drives resistance to RAF inhibition through MAP kinase pathway reactivation. *Nature* **468**: 968–972.
- Jolly MK, Jia D, Boareto M, Mani SA, Pienta KJ, Ben-Jacob E, Levine H. 2015. Coupling the modules of EMT and stemness: A tunable “stemness window” model. *Oncotarget* **6**: 25161–25174.
- Kalluri R, Weinberg RA. 2009. The basics of epithelial–mesenchymal transition. *J Clin Invest* **119**: 1420–1428.
- Kang Y, Siegel PM, Shu W, Drobnjak M, Kakonen SM, Córdón-Cardo C, Guise TA, Massagué J. 2003. A multigenic program mediating breast cancer metastasis to bone. *Cancer Cell* **3**: 537–549.
- Kang Y, He W, Tulley S, Gupta GP, Serganova I, Chen CR, Manova-Todorova K, Blasberg R, Gerald WL, Massagué J. 2005. Breast cancer bone metastasis mediated by the Smad tumor suppressor pathway. *Proc Natl Acad Sci* **102**: 13909–13914.
- Kano MR, Bae Y, Iwata C, Morishita Y, Yashiro M, Oka M, Fujii T, Komuro A, Kiyono K, Kaminishi M, et al. 2007. Improvement of cancer-targeting therapy, using nanocarriers for intractable solid tumors by inhibition of TGF- β signaling. *Proc Natl Acad Sci* **104**: 3460–3465.
- Kasid A, Bell GI, Director EP. 1988. Effects of transforming growth factor- β on human lymphokine-activated killer cell precursors. Autocrine inhibition of cellular proliferation and differentiation to immune killer cells. *J Immunol* **141**: 690–698.
- Kawasaki K, Freimuth J, Meyer DS, Lee MM, Tochimoto-Okamoto A, Benzinou M, Clermont FF, Wu G, Roy R, Letteboer TG, et al. 2014. Genetic variants of Adam17 differentially regulate TGF- β signaling to modify vascular pathology in mice and humans. *Proc Natl Acad Sci* **111**: 7723–7728.
- Kim SJ, Denhez F, Kim KY, Holt JT, Sporn MB, Roberts AB. 1989. Activation of the second promoter of the transforming growth factor- β 1 gene by transforming growth factor- β 1 and phorbol ester occurs through the same target sequences. *J Biol Chem* **264**: 19373–19378.
- Kim S, Buchlis G, Fridlender ZG, Sun J, Kapoor V, Cheng G, Haas A, Cheung HK, Zhang X, Corbley M, et al. 2008. Systemic blockade of transforming growth factor- β signaling augments the efficacy of immunogene therapy. *Cancer Res* **68**: 10247–10256.
- Kondo M, Cubillo E, Tobiume K, Shirakihara T, Fukuda N, Suzuki H, Shimizu K, Takehara K, Cano A, Saitoh M, et al. 2004. A role for Id in the regulation of TGF- β -induced epithelial-mesenchymal transdifferentiation. *Cell Death Differ* **11**: 1092–1101.
- Kong FM, Anscher MS, Murase T, Abbott BD, Iglehart JD, Jirtle RL. 1995. Elevated plasma transforming growth factor- β 1 levels in breast cancer patients decrease after surgical removal of the tumor. *Ann Surg* **222**: 155–162.
- Koo E, Henderson MA, Dwyer M, Skandarajah AR. 2015. Management and prevention of breast cancer after radiation to the chest for childhood, adolescent, and young adulthood malignancy. *Ann Surg Oncol* **22**: 545–551.
- Kovacs RJ, Maldonado G, Azaro A, Fernández MS, Romero FL, Sepulveda-Sánchez JM, Corretti M, Carducci M, Dolan M, Gueorguieva I, et al. 2015. Cardiac safety of TGF- β receptor I kinase inhibitor LY2157299 monohydrate in cancer patients in a first-in-human dose study. *Cardiovas Toxicol* **15**: 309–323.
- Kozlow W, Guise TA. 2005. Breast cancer metastasis to bone: Mechanisms of osteolysis and implications for therapy. *J Mammary Gland Biol Neoplasia* **10**: 169–180.

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- Krasagakis K, Tholke D, Farthmann B, Eberle J, Mansmann U, Orfanos CE. 1998. Elevated plasma levels of transforming growth factor (TGF)- β 1 and TGF- β 2 in patients with disseminated malignant melanoma. *Br J Cancer* **77**: 1492–1494.
- Kretzschmar M, Doody J, Timokhina I, Massagué J. 1999. A mechanism of repression of TGF- β /Smad signaling by oncogenic Ras. *Genes Dev* **13**: 804–816.
- Kulkarni AB, Huh CG, Becker D, Geiser A, Lyght M, Flanders KC, Roberts AB, Sporn MB, Ward JM, Karlsson S. 1993. Transforming growth factor β 1 null mutation in mice causes excessive inflammatory response and early death. *Proc Natl Acad Sci* **90**: 770–774.
- Kuppner MC, Hamou MF, Bodmer S, Fontana A, De Tribolet N. 1988. The glioblastoma-derived T-cell suppressor factor/transforming growth factor β 2 inhibits the generation of lymphokine-activated killer (LAK) cells. *Int J Cancer* **42**: 562–567.
- Lacher MD, Tiirikainen MI, Saunier EE, Christian C, Anders M, Oft M, Balmain A, Akhurst RJ, Korn WM. 2006. Transforming growth factor- β receptor inhibition enhances adenoviral infectability of carcinoma cells via up-regulation of coxsackie and adenovirus receptor in conjunction with reversal of epithelial–mesenchymal transition. *Cancer Res* **66**: 1648–1657.
- Lacouture ME, O'Reilly K, Rosen N, Solit DB. 2012. Induction of cutaneous squamous cell carcinomas by RAF inhibitors: Cause for concern? *J Clin Onc* **30**: 329–330.
- Lacouture ME, Morris JC, Lawrence DP, Tan AR, Olencki TE, Shapiro GI, Dezube BJ, Berzofsky JA, Hsu FJ, Guitart J. 2015. Cutaneous keratoacanthomas/squamous cell carcinomas associated with neutralization of transforming growth factor β by the monoclonal antibody fresolimumab (GC1008). *Cancer Immunol Immunother* **64**: 437–446.
- Lammerts E, Roswall P, Sundberg C, Gotwals PJ, Kotliansky VE, Reed RK, Heldin NE, Rubin K. 2002. Interference with TGF- β 1 and - β 3 in tumor stroma lowers tumor interstitial fluid pressure independently of growth in experimental carcinoma. *Int J Cancer* **102**: 453–462.
- Lamouille S, Derynck R. 2009. Oncogene and tumour suppressor: The two faces of SnoN. *EMBO J* **28**: 3459–3460.
- Laouar Y, Sutterwala FS, Gorelik L, Flavell RA. 2005. Transforming growth factor- β controls T helper type 1 cell development through regulation of natural killer cell interferon- γ . *Nat Immunol* **6**: 600–607.
- Laping NJ, Everitt JJ, Frazier KS, Burgert M, Portis MJ, Cadacio C, Gold LI, Walker CL. 2007. Tumor-specific efficacy of transforming growth factor- β RI inhibition in Eker rats. *Clin Cancer Res* **13**: 3087–3099.
- Laverty HG, Wakefield LM, Ocleston NL, O'Kane S, Ferguson MW. 2009. TGF- β 3 and cancer: A review. *Cytokine Growth Factor Rev* **20**: 305–317.
- Leitlein J, Aulwurm S, Waltereit R, Naumann U, Wagenknecht B, Garten W, Weller M, Platten M. 2001. Processing of immunosuppressive pro-TGF- β 1,2 by human glioblastoma cells involves cytoplasmic and secreted furin-like proteases. *J Immunol* **166**: 7238–7243.
- Levental KR, Yu H, Kass L, Lakins JN, Egeblad M, Erler JT, Fong SF, Csiszar K, Giaccia A, Weninger W, et al. 2009. Matrix crosslinking forces tumor progression by enhancing integrin signaling. *Cell* **139**: 891–906.
- Li MO, Flavell RA. 2008. TGF- β : A master of all T cell trades. *Cell* **134**: 392–404.
- Li J, Foitzik K, Calautti E, Baden H, Doetschman T, Dotto GP. 1999. TGF- β 3, but not TGF- β 1, protects keratinocytes against 12-O-tetradecanoylphorbol-13-acetate-induced cell death in vitro and in vivo. *J Biol Chem* **274**: 4213–4219.
- Li X, Lewis MT, Huang J, Gutierrez C, Osborne CK, Wu MF, Hilsenbeck SG, Pavlick A, Zhang X, Chamness GC, et al. 2008. Intrinsic resistance of tumorigenic breast cancer cells to chemotherapy. *J Natl Cancer Inst* **100**: 672–679.
- Lipson EJ, Sharfman WH, Drake CG, Wollner I, Taube JM, Anders RA, Xu H, Yao S, Pons A, Chen L, et al. 2013. Durable cancer regression off-treatment and effective re-induction therapy with an anti-PD-1 antibody. *Clin Cancer Res* **19**: 462–468.
- Lucke CD, Philpott A, Metcalfe JC, Thompson AM, Hughes-Davies L, Kemp PR, Hesketh R. 2001. Inhibiting mutations in the transforming growth factor β type 2 receptor in recurrent human breast cancer. *Cancer Res* **61**: 482–485.
- Maker AV, Yang JC, Sherry RM, Topalian SL, Kammula US, Royal RE, Hughes M, Yellin MJ, Haworth LR, Levy C, et al. 2006. Inpatient dose escalation of anti-CTLA-4 antibody in patients with metastatic melanoma. *J Immunother* **29**: 455–463.
- Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M, et al. 2008. The epithelial–mesenchymal transition generates cells with properties of stem cells. *Cell* **133**: 704–715.
- Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. 2002. Macrophage polarization: Tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol* **23**: 549–555.
- Margadant C, Sonnenberg A. 2010. Integrin-TGF- β crosstalk in fibrosis, cancer and wound healing. *EMBO Rep* **11**: 97–105.
- Matsuda H, Fukuda N, Ueno T, Katakawa M, Wang X, Watanabe T, Matsui S, Aoyama T, Saito K, Bando T, et al. 2011. Transcriptional inhibition of progressive renal disease by gene silencing pyrrole-imidazole polyamide targeting of the transforming growth factor- β 1 promoter. *Kidney Int* **79**: 46–56.
- Mi LZ, Brown CT, Gao Y, Tian Y, Le VQ, Walz T, Springer TA. 2015. Structure of bone morphogenetic protein 9 pro-complex. *Proc Natl Acad Sci* **112**: 3710–15.
- Miyazono K. 2012. Tumour promoting functions of TGF- β in CML-initiating cells. *J Biochem* **152**: 383–385.
- Mohammad KS, Javelaud D, Fournier PG, Niewolna M, McKenna CR, Peng XH, Duong V, Dunn LK, Mauviel A, Guise TA. 2011. TGF- β -RI kinase inhibitor SD-208 reduces the development and progression of melanoma bone metastases. *Cancer Res* **71**: 175–184.
- Morris JC, Shapiro GI, Tan AR, Lawrence DP, Olencki TE, Dezube BJ, et al., eds. 2008. Phase I/II study of GC1008: A human anti-transforming growth factor-beta (TGF β) monoclonal antibody in patients with advanced malignant melanoma (MM) or renal cell carcinoma (RCC). ASCO Annual Meeting.
- Morris JC, Tan AR, Olencki TE, Shapiro GI, Dezube BJ, Reiss M, Hsu FJ, Berzofsky JA, Lawrence DP. 2014. Phase I study of GC1008 (fresolimumab): A human anti-trans-

- forming growth factor- β (TGF β) monoclonal antibody in patients with advanced malignant melanoma or renal cell carcinoma. *PLoS ONE* **9**: e90353.
- Munger JS, Harpel JG, Gleizes PE, Mazzieri R, Nunes I, Rifkin DB. 1997. Latent transforming growth factor- β : Structural features and mechanisms of activation. *Kidney Int* **51**: 1376–1382.
- Munger JS, Huang X, Kawakatsu H, Griffiths MJ, Dalton SL, Wu J, Pittet JF, Kaminski N, Garat C, Matthey MA, et al. 1999. The integrin α v β 6 binds and activates latent TGF- β 1: A mechanism for regulating pulmonary inflammation and fibrosis. *Cell* **96**: 319–328.
- Naka K, Hoshii T, Muraguchi T, Tadokoro Y, Ooshio T, Kondo Y, Nakao S, Motoyama N, Hirao A. 2010. TGF- β -FOXO signalling maintains leukaemia-initiating cells in chronic myeloid leukaemia. *Nature* **463**: 676–680.
- Naka K, Ishihara K, Jomen Y, Jin CH, Kim DH, Gu YK, Jeong ES, Li S, Krause DS, Kim DW, et al. 2016. Novel oral transforming growth factor- β signaling inhibitor EW-7197 eradicates CML-initiating cells. *Cancer Sci* **107**: 140–148.
- Nazarian R, Shi H, Wang Q, Kong X, Koya RC, Lee H, Chen Z, Lee MK, Attar N, Sazegar H, et al. 2010. Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-RAS upregulation. *Nature* **468**: 973–977.
- Nemunaitis J, Murray N. 2006. Immune-modulating vaccines in non-small cell lung cancer. *J Thoracic Oncol* **1**: 756–761.
- Nemunaitis J, Dillman RO, Schwarzenberger PO, Senzer N, Cunningham C, Cutler J, Tong A, Kumar P, Pappen B, Hamilton C, et al. 2006. Phase II study of belagenpumatucel-L, a transforming growth factor β -2 antisense gene-modified allogeneic tumor cell vaccine in non-small-cell lung cancer. *J Clin Oncol* **24**: 4721–4730.
- Nemunaitis J, Nemunaitis M, Senzer N, Snitz P, Bedell C, Kumar P, Pappen B, Maples PB, Shawler D, Fakhrizadeh H. 2009. Phase II trial of belagenpumatucel-L, a TGF- β 2 antisense gene modified allogeneic tumor vaccine in advanced non small cell lung cancer (NSCLC) patients. *Cancer Gene Ther* **16**: 620–624.
- Ng MR, Brugge JS. 2009. A stiff blow from the stroma: Collagen crosslinking drives tumor progression. *Cancer Cell* **16**: 455–457.
- Oft M, Akhurst RJ, Balmain A. 2002. Metastasis is driven by sequential elevation of H-ras and Smad2 levels. *Nat Cell Biol* **4**: 487–494.
- Ogino S, Kawasaki T, Ogawa A, Kirkner GJ, Loda M, Fuchs CS. 2007. TGFBR2 mutation is correlated with CpG island methylator phenotype in microsatellite instability-high colorectal cancer. *Human Pathol* **38**: 614–620.
- Oshimori N, Oristian D, Fuchs E. 2015. TGF- β promotes heterogeneity and drug resistance in squamous cell carcinoma. *Cell* **160**: 963–976.
- Ota T, Fujii M, Sugizaki T, Ishii M, Miyazawa K, Aburatani H, Miyazono K. 2002. Targets of transcriptional regulation by two distinct type I receptors for transforming growth factor- β in human umbilical vein endothelial cells. *J Cell Physiol* **193**: 299–318.
- Padua D, Massagué J. 2009. Roles of TGF- β in metastasis. *Cell Res* **19**: 89–102.
- Papageorgis P, Lambert AW, Ozturk S, Gao F, Pan H, Manne U, Alekseyev YO, Thiagalingam A, Abdolmaleky HM, Lenburg M, et al. 2010. Smad signaling is required to maintain epigenetic silencing during breast cancer progression. *Cancer Res* **70**: 968–978.
- Park J, Wrzesinski SH, Stern E, Look M, Criscione J, Ragheb R, Jay SM, Demento SL, Agawu A, Licona Limon P, et al. 2012. Combination delivery of TGF- β inhibitor and IL-2 by nanoscale liposomal polymeric gels enhances tumour immunotherapy. *Nat Mat* **11**: 895–905.
- Parsons R, Myeroff LL, Liu B, Willson JK, Markowitz SD, Kinzler KW, Vogelstein B. 1995. Microsatellite instability and mutations of the transforming growth factor- β type II receptor gene in colorectal cancer. *Cancer Res* **55**: 5548–5550.
- Peterson AJ, O'Connor MB. 2013. Activin receptor inhibition by Smad2 regulates *Drosophila* wing disc patterning through BMP-response elements. *Development* **140**: 649–659.
- Piek E, Ju WJ, Heyer J, Escalante-Alcalde D, Stewart CL, Weinstein M, Deng C, Kuchlerlapati R, Bottinger EP, Roberts AB. 2001. Functional characterization of transforming growth factor β signaling in Smad2- and Smad3-deficient fibroblasts. *J Biol Chem* **276**: 19945–19953.
- Portella G, Cumming SA, Liddell J, Cui W, Ireland H, Akhurst RJ, Balmain A. 1998. Transforming growth factor β is essential for spindle cell conversion of mouse skin carcinoma in vivo: Implications for tumor invasion. *Cell Growth Diff* **9**: 393–404.
- Rabbani ZN, Anscher MS, Zhang X, Chen L, Samulski TV, Li CY, Vujaskovic Z. 2003. Soluble TGF- β type II receptor gene therapy ameliorates acute radiation-induced pulmonary injury in rats. *Int J Radiat Oncol Biol Phys* **57**: 563–572.
- Ramesh S, Wildev GM, Howe PH. 2009. Transforming growth factor β (TGF- β)-induced apoptosis: The rise and fall of Bim. *Cell Cycle* **8**: 11–17.
- Ray BN, Lee NY, How T, Blobe GC. 2010. ALK5 phosphorylation of the endoglin cytoplasmic domain regulates Smad1/5/8 signaling and endothelial cell migration. *Carcinogenesis* **31**: 435–441.
- Rice LM, Padilla CM, McLaughlin SR, Mathes A, Ziemek J, Goummih S, Nakerakanti S, York M, Farina G, Whitfield ML, et al. 2015. Fresolimumab treatment decreases biomarkers and improves clinical symptoms in systemic sclerosis patients. *J Clin Invest* **125**: 2795–2807.
- Roberts AB, Wakefield LM. 2003. The two faces of transforming growth factor β in carcinogenesis. *Proc Natl Acad Sci* **100**: 8621–8623.
- Robinson PN, Arteaga-Solis E, Baldock C, Collod-Bérout G, Booms P, De Paepe A, Dietz HC, Guo G, Handford PA, Judge DP, et al. 2006. The molecular genetics of Marfan syndrome and related disorders. *J Med Genet* **43**: 769–787.
- Rodón J, Carducci M, Sepulveda-Sánchez JM, Azaro A, Calvo E, Seoane J, Braña I, Sicart E, Gueorguieva I, Clevverly A, et al. 2015a. Pharmacokinetic, pharmacodynamic and biomarker evaluation of transforming growth factor- β receptor I kinase inhibitor, galunisertib, in phase I study in patients with advanced cancer. *Invest New Drugs* **33**: 357–370.

R.J. Akhurst

- Rodón J, Carducci MA, Sepulveda-Sánchez JM, Azaro A, Calvo E, Seoane J, Braña I, Sicart E, Gueorguieva I, Clevverly AL, et al. 2015b. First-in-human dose study of the novel transforming growth factor- β receptor I kinase inhibitor LY2157299 monohydrate in patients with advanced cancer and glioma. *Clin Cancer Res* **21**: 553–560.
- Rubtsov YP, Rudensky AY. 2007. TGF- β signalling in control of T-cell-mediated self-reactivity. *Nat Rev Immunol* **7**: 443–453.
- Salnikov AV, Roswall P, Sundberg C, Gardner H, Heldin NE, Rubin K. 2005. Inhibition of TGF- β modulates macrophages and vessel maturation in parallel to a lowering of interstitial fluid pressure in experimental carcinoma. *Lab Invest* **85**: 512–521.
- Salvo E, Garasa S, Dotor J, Morales X, Pelaez R, Altevoigt P, Rouzaut A. 2014. Combined targeting of TGF- β 1 and integrin β 3 impairs lymph node metastasis in a mouse model of non-small-cell lung cancer. *Mol Cancer* **13**: 112.
- Sato K, Kawasaki H, Nagayama H. 2000. TGF- β 1 reciprocally controls chemotaxis of human peripheral blood monocyte-derived dendritic cells via chemokine receptors. *J Immunol* **164**: 2285–2295.
- Sawyer JS, Anderson BD, Beight DW, Campbell RM, Jones ML, Herron DK, Lampe JW, McCowan JR, McMillen WT, Mort N, et al. 2003. Synthesis and activity of new aryl- and heteroaryl-substituted pyrazole inhibitors of the transforming growth factor- β type I receptor kinase domain. *J Med Chem* **46**: 3953–3956.
- Schlingensiepen KH, Jaschinski F, Lang SA, Moser C, Geissler EK, Schlitt HJ, Kielmanowicz M, Schneider A. 2011. Transforming growth factor- β 2 gene silencing with trabedersen (AP 12009) in pancreatic cancer. *Cancer Sci* **102**: 1193–1200.
- Schmierer B, Hill CS. 2007. TGF- β –SMAD signal transduction: Molecular specificity and functional flexibility. *Nat Rev Mol Cell Biol* **8**: 970–982.
- Schultz-Cherry S, Ribeiro S, Gentry L, Murphy-Ullrich JE. 1994. Thrombospondin binds and activates the small and large forms of latent transforming growth factor- β in a chemically defined system. *J Biol Chem* **269**: 26775–26782.
- Sepulveda-Sánchez J, Ramos A, Hilario A, DE Velasco G, Castellano D, Garcia DE LA Torre M, Rodon J, Lahn ME. 2015. Brain perfusion and permeability in patients with advanced, refractory glioblastoma treated with lomustine and the transforming growth factor- β receptor I kinase inhibitor LY2157299 monohydrate. *Oncol Lett* **9**: 2442–2448.
- Sequist LV, Martins RG, Spigel D, Grunberg SM, Spira A, Jänne PA, Joshi VA, McCollum D, Evans TL, Muzikansky A, et al. 2008. First-line gefitinib in patients with advanced non-small-cell lung cancer harboring somatic EGFR mutations. *J Clin Oncol* **26**: 2442–2449.
- Sergina NV, Rausch M, Wang D, Blair J, Hann B, Shokat KM, Moasser MM. 2007. Escape from HER-family tyrosine kinase inhibitor therapy by the kinase-inactive HER3. *Nature* **445**: 437–441.
- Sharma SV, Lee DY, Li B, Quinlan MP, Takahashi F, Maheswaran S, McDermott U, Azizian N, Zou L, Fischbach MA, et al. 2010. A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. *Cell* **141**: 69–80.
- Shi Y, Massagué J. 2003. Mechanisms of TGF- β signaling from cell membrane to the nucleus. *Cell* **113**: 685–700.
- Shi M, Zhu J, Wang R, Chen X, Mi L, Walz T, Springer TA. 2011. Latent TGF- β structure and activation. *Nature* **474**: 343–349.
- Shim KS, Kim KH, Han WS, Park EB. 1999. Elevated serum levels of transforming growth factor- β 1 in patients with colorectal carcinoma: Its association with tumor progression and its significant decrease after curative surgical resection. *Cancer* **85**: 554–561.
- Shinto O, Yashiro M, Kawajiri H, Shimizu K, Shimizu T, Miwa A, Hirakawa K. 2010. Combination effect of a TGF- β receptor kinase inhibitor with 5-FU analog S1 on lymph node metastasis of scirrhous gastric cancer in mice. *Cancer Sci* **101**: 1846–1852.
- Shipitsin M, Campbell LL, Argani P, Weremowicz S, Bloushtain-Qimron N, Yao J, Nikolskaya T, Serebryskaya T, Beroukhi R, Hu M, et al. 2007. Molecular definition of breast tumor heterogeneity. *Cancer Cell* **11**: 259–273.
- Shull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RJ, Yin M, Allen R, Sidman C, Proetzel G, Calvin D, et al. 1992. Targeted disruption of the mouse transforming growth factor- β 1 gene results in multifocal inflammatory disease. *Nature* **359**: 693–699.
- Siegel PM, Shu W, Massagué J. 2003. Mad upregulation and Id2 repression accompany transforming growth factor (TGF)- β -mediated epithelial cell growth suppression. *J Biol Chem* **278**: 35444–35450.
- Singh A, Settleman J. 2010. EMT, cancer stem cells and drug resistance: An emerging axis of evil in the war on cancer. *Oncogene* **29**: 4741–4751.
- Son JY, Park SY, Kim SJ, Lee SJ, Park SA, Kim MJ, Kim SW, Kim DK, Nam JS, Sheen YY. 2014. EW-7197, a novel ALK-5 kinase inhibitor, potentially inhibits breast to lung metastasis. *Mol Cancer Therap* **13**: 1704–1716.
- Sorrentino A, Thakur N, Grimsby S, Marcusson A, von Bulow V, Schuster N, Zhang S, Heldin CH, Landström M. 2008. The type I TGF- β receptor engages TRAF6 to activate TAK1 in a receptor kinase-independent manner. *Nat Cell Biol* **10**: 1199–1207.
- Spain L, Diem S, Larkin J. 2016. Management of toxicities of immune checkpoint inhibitors. *Cancer Treat Rev* **44**: 51–60.
- Stampfer MR, Yaswen P, Alhadeff M, Hosoda J. 1993. TGF β induction of extracellular matrix associated proteins in normal and transformed human mammary epithelial cells in culture is independent of growth effects. *J Cell Physiol* **155**: 210–221.
- Stockis J, Colau D, Coulie PG, Lucas S. 2009. Membrane protein GARP is a receptor for latent TGF- β on the surface of activated human Treg. *Eur J Immunol* **39**: 3315–3322.
- Sundar R, Gudey SK, Heldin CH, Landström M. 2015. TRAF6 promotes TGF β -induced invasion and cell-cycle regulation via Lys63-linked polyubiquitination of Lys178 in TGF β type I receptor. *Cell Cycle* **14**: 554–565.
- Suzuki E, Kapoor V, Cheung HK, Ling LE, Delong PA, Kaiser LR, Albelda SM. 2004. Soluble type II transforming growth factor- β receptor inhibits established murine malignant mesothelioma tumor growth by augmenting host antitumor immunity. *Clin Cancer Res* **10**: 5907–5918.

- Takeuchi K, Abe M, Hiasa M, Oda A, Amou H, Kido S, Harada T, Tanaka O, Miki H, Nakamura S, et al. 2010. TGF- β inhibition restores terminal osteoblast differentiation to suppress myeloma growth. *PLoS ONE* **5**: e9870.
- Tampe D, Zeisberg M. 2014. Potential approaches to reverse or repair renal fibrosis. *Nat Rev Nephrol* **10**: 226–237.
- Tang B, Yoo N, Vu M, Mamura M, Nam JS, Ooshima A, Du Z, Desprez PY, Anver MR, Michalowska AM, et al. 2007. Transforming growth factor- β can suppress tumorigenesis through effects on the putative cancer stem or early progenitor cell and committed progeny in a breast cancer xenograft model. *Cancer Res* **67**: 8643–8652.
- TCGA Network T. 2015. Comprehensive genomic characterization of head and neck squamous cell carcinomas. *Nature* **517**: 576–582.
- Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, et al. 2000. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* **92**: 205–216.
- Topalian SL, Drake CG, Pardoll DM. 2012. Targeting the PD-1/B7-H1(PD-L1) pathway to activate anti-tumor immunity. *Curr Opin Immunol* **24**: 207–212.
- Tran DQ, Andersson J, Wang R, Ramsey H, Unutmaz D, Shevach EM. 2009. GARP (LRRC32) is essential for the surface expression of latent TGF- β on platelets and activated FOXP3⁺ regulatory T cells. *Proc Natl Acad Sci* **106**: 13445–13450.
- Triplett TA, Tucker CG, Triplett KC, Alderman Z, Sun L, Ling LE, Akporiaye ET, Weinberg AD. 2015. STAT3 signaling is required for optimal regression of large established tumors in mice treated with anti-OX40 and TGF β receptor blockade. *Cancer Immunol Res* **3**: 526–535.
- Tuma RS. 2008. New response criteria proposed for immunotherapies. *J Natl Cancer Inst* **100**: 1280–1281.
- Valle L, Serena-Acedo T, Liyanarachchi S, Hampel H, Comeras I, Li Z, Zeng Q, Zhang HT, Pennison MJ, Sadim M, et al. 2008. Germline allele-specific expression of *TGFBR1* confers an increased risk of colorectal cancer. *Science* **321**: 1361–1365.
- Vallieres L. 2009. Trabectedin, a TGF β 2-specific antisense oligonucleotide for the treatment of malignant gliomas and other tumors overexpressing TGF β 2. *IDrugs* **12**: 445–453.
- Van Aarsen LA, Leone DR, Ho S, et al. 2008. Antibody-mediated blockade of integrin $\alpha\beta 6$ inhibits tumor progression in vivo by a transforming growth factor- β -regulated mechanism. *Cancer Res* **68**: 561–570.
- Van Der Jeught K, Joe PT, Bialkowski L, Heirman C, Daszkiewicz L, Liechtenstein T, Escors D, Thielemans K, Breckpot K. 2014. Intratumoral administration of mRNA encoding a fusokine consisting of IFN- β and the ectodomain of the TGF- β receptor II potentiates antitumor immunity. *Oncotarget* **5**: 10100–10113.
- Vanpouille-Box C, Diamond JM, Pilonis KA, Zavadil J, Babb JS, Formenti SC, Barcellos-Hoff MH, Demaria S. 2015. TGF β is a master regulator of radiation therapy-induced antitumor immunity. *Cancer Res* **75**: 2232–2242.
- Vinogradova M, Gehling VS, Gustafson A, Arora S, Tindell CA, Wilson C, Williamson KE, Guler GD, Gangurde P, Manieri W, et al. 2016. An inhibitor of KDM5 demethylases reduces survival of drug-tolerant cancer cells. *Nat Chem Biol* **12**: 531–538.
- Voulgari A, Pintzas A. 2009. Epithelial–mesenchymal transition in cancer metastasis: Mechanisms, markers and strategies to overcome drug resistance in the clinic. *Biochim Biophys Acta* **1796**: 75–90.
- Walker RA, Dearing SJ. 1992. Transforming growth factor β 1 in ductal carcinoma in situ and invasive carcinomas of the breast. *Eur J Cancer* **28**: 641–644.
- Wallace A, Kapoor V, Sun J, Mrass P, Weninger W, Heitjan DF, June C, Kaiser LR, Ling LE, Albelda SM. 2008. Transforming growth factor- β receptor blockade augments the effectiveness of adoptive T-cell therapy of established solid cancers. *Clin Cancer Res* **14**: 3966–3974.
- Washio H, Fukuda N, Matsuda H, Nagase H, Watanabe T, Matsumoto Y, Terui T. 2011. Transcriptional inhibition of hypertrophic scars by a gene silencer, pyrrole-imidazole polyamide, targeting the TGF- β 1 promoter. *J Invest Derm* **131**: 1987–1995.
- Weigert C, Sauer U, Brodbeck K, Pfeiffer A, Haring HU, Schleicher ED. 2000. AP-1 proteins mediate hyperglycemia-induced activation of the human TGF- β 1 promoter in mesangial cells. *J Am Soc Nephrol* **11**: 2007–2016.
- Wick W, Weller M. 2011. Trabectedin to target transforming growth factor- β : When the journey is not the reward. *Neuro Oncol* **13**: 559–560.
- Wicks SJ, Lui S, Abdel-Wahab N, Mason RM, Chantry A. 2000. Inactivation of Smad-transforming growth factor β signaling by Ca^{2+} -calmodulin-dependent protein kinase II. *Mol Cell Biol* **20**: 8103–8111.
- Wu CY, Roybal KT, Puchner EM, Onuffer J, Lim WA. 2015. Remote control of therapeutic T cells through a small molecule-gated chimeric receptor. *Science* **350**: aab4077.
- Xie W, Mertens JC, Reiss DJ, Rimm DL, Camp RL, Haffty BG, Reiss M. 2002. Alterations of Smad signaling in human breast carcinoma are associated with poor outcome: A tissue microarray study. *Cancer Res* **62**: 497–505.
- Yakymovych I, ten Dijke P, Heldin CH, Souchelnytskyi S. 2001. Regulation of Smad signaling by protein kinase C. *FASEB J* **15**: 553–555.
- Yamaguchi Y, Tsumura H, Miwa M, Inaba K. 1997. Contrasting effects of TGF- β 1 and TNF- α on the development of dendritic cells from progenitors in mouse bone marrow. *Stem Cells* **15**: 144–153.
- Yamashita S, Takahashi S, McDonnell N, Watanabe N, Niwa T, Hosoya K, Tsujino Y, Shirai T, Ushijima T. 2008. Methylation silencing of transforming growth factor- β receptor type II in rat prostate cancers. *Cancer Res* **68**: 2112–2121.
- Yang Z, Mu Z, Dabovic B, Jurukovski V, Yu D, Sung J, Xiong X, Munger JS. 2007. Absence of integrin-mediated TGF β 1 activation in vivo recapitulates the phenotype of TGF β 1-null mice. *J Cell Biol* **176**: 787–793.
- Yao EH, Fukuda N, Ueno T, Matsuda H, Nagase H, Matsumoto Y, Sugiyama H, Matsumoto K. 2009. A pyrrole-

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imidazole polyamide targeting transforming growth factor- β 1 inhibits restenosis and preserves endothelialization in the injured artery. *Cardiovasc Res* **81**: 797–804.

Yin JJ, Selander K, Chirgwin JM, Dallas M, Grubbs BG, Wieser R, Massagué J, Mundy GR, Guise TA. 1999. TGF- β signaling blockade inhibits PTHrP secretion by breast cancer cells and bone metastases development. *J Clin Invest* **103**: 197–206.

Yingling JM, Blanchard KL, Sawyer JS. 2004. Development of TGF- β signalling inhibitors for cancer therapy. *Nat Rev Drug Discov* **3**: 1011–1022.

Yoon JH, Jung SM, Park SH, Kato M, Yamashita T, Lee IK, Sudo K, Nakae S, Han JS, Kim OH, et al. 2013. Activin receptor-like kinase 5 inhibition suppresses mouse melanoma by ubiquitin degradation of Smad4, thereby de-repressing eomesodermin in cytotoxic T lymphocytes. *EMBO Mol Med* **5**: 1720–1739.

Yun CH, Mengwasser KE, Toms AV, Woo MS, Greulich H, Wong KK, Meyerson M, Eck MJ. 2008. The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. *Proc Natl Acad Sci* **105**: 2070–2075.

Zhang Z, Wang Y, Vikis HG, Johnson L, Liu G, Li J, Anderson MW, Sills RC, Hong HL, Devereux TR, et al. 2001. Wildtype *Kras2* can inhibit lung carcinogenesis in mice. *Nat Genet* **29**: 25–33.

Zhang M, Kleber S, Röhrich M, Timke C, Han N, Tuettenberg J, Martin-Villalba A, Debus J, Peschke P, Wirkner U, et al. 2011. Blockade of TGF- β signaling by the TGF β R-I kinase inhibitor LY2109761 enhances radiation response and prolongs survival in glioblastoma. *Cancer Res* **71**: 7155–7167.

Zhong Z, Carroll KD, Policarpio D, Osborn C, Gregory M, Bassi R, Jimenez X, Prewett M, Liebisch G, Persaud K, et al. 2010. Anti-transforming growth factor β receptor II antibody has therapeutic efficacy against primary tumor growth and metastasis through multieffects on cancer, stroma, and immune cells. *Clin Cancer Res* **16**: 1191–1205.

Zhou M, Bracci PM, McCoy LS, Hsuang G, Wiemels JL, Rice T, Zheng S, Kelsey KT, Wensch MR, Wiencke JK. 2015. Serum macrophage-derived chemokine/CCL22 levels are associated with glioma risk, CD4 T cell lymphopenia and survival time. *Int J Cancer* **137**: 826–836.

Zhu X, Wang L, Zhang B, Li J, Dou X, Zhao RC. 2011. TGF- β 1-induced PI3K/Akt/NF κ B/MMP9 signalling pathway is activated in Philadelphia chromosome-positive chronic myeloid leukaemia hemangioblasts. *J Biochem* **149**: 405–414.

Zuber P, Kupfer MC, De Tribolet N. 1988. Transforming growth factor- β 2 down-regulates HLA-DR antigen expression on human malignant glioma cells. *Eur J Immunol* **18**: 1623–1626.

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