Clinical & Experimental Metastasis (2021) 38:139–161
https://doi.org/10.1007/s10585-021-10077-z

REVIEW

TGFβ signaling networks in ovarian cancer progression and plasticity

Asha Kumari1 · Zainab Shonibare1 · Mehri Monavarian1 · Rebecca C. Arend2 · Nam Y. Lee3 · Gareth J. Inman4 · Karthikeyan Mythreye1

Received: 19 October 2020 / Accepted: 3 February 2021 / Published online: 15 February 2021
© The Author(s) 2021

Abstract
Epithelial ovarian cancer (EOC) is a leading cause of cancer-related death in women. Late-stage diagnosis with significant tumor burden, accompanied by recurrence and chemotherapy resistance, contributes to this poor prognosis. These morbidities are known to be tied to events associated with epithelial-mesenchymal transition (EMT) in cancer. During EMT, localized tumor cells alter their polarity, cell–cell junctions, cell–matrix interactions, acquire motility and invasiveness and an exaggerated potential for metastatic spread. Key triggers for EMT include the Transforming Growth Factor-β (TGFβ) family of growth factors which are actively produced by a wide array of cell types within a specific tumor and metastatic environment. Although TGFβ can act as either a tumor suppressor or promoter in cancer, TGFβ exhibits its pro-tumorigenic functions at least in part via EMT. TGFβ regulates EMT both at the transcriptional and post-transcriptional levels as outlined here. Despite recent advances in TGFβ based therapeutics, limited progress has been seen for ovarian cancers that are in much need of new therapeutic strategies. Here, we summarize and discuss several recent insights into the underlying signaling mechanisms of the TGFβ isoforms in EMT in the unique metastatic environment of EOCs and the current therapeutic interventions that may be relevant.

Keywords EMT · TGFβ · Ovarian cancer · Tumor microenvironment · Metastasis

Abbreviations
TGFβ Transforming growth factor beta

Introduction

Epithelial-mesenchymal transition (EMT) is a mechanism of trans-differentiation of epithelial cells into mesenchymal cells, wherein polarized epithelial cells alter their contacts with their neighboring cells, basement membrane and the surrounding tissues [1]. EMT is critical for embryogenesis and organ development as first reported in 1995 and since has been studied in great detail in both physiological and pathological conditions including wound healing, chronic disease with fibrotic changes and cancer progression [2, 3]. The presence of mesenchymal cells is associated with metastatic dissemination in cancer [4] as these cells can alter the extracellular matrix (ECM), and are more invasive and motile. On the opposite end of EMT is mesenchymal to epithelial transition (MET), which also occurs during development, wound healing, fibrosis and in cancer, where it may support metastatic outgrowth at distant sites [5]. Between EMT and MET, incomplete or partial EMT may occur, leading to hybrid states with simultaneous expression of epithelial (E) and mesenchymal (M) markers (E/M), observed in multiple cancer types [6, 7]. These intermediate E/M forms can differentiate into either mesenchymal cell types via EMT or revert back to an epithelial state by MET mechanisms providing a spectrum of plasticity opportunities to the cells [8]. The molecular and transcriptional networks regulating EMT are central to the process and are tightly regulated during both development and disease progression [2, 3]. Key

1 Division of Molecular and Cellular Pathology, Department of Pathology, University of Alabama at Birmingham, WTI 320B, 1824 Sixth Avenue South, Birmingham, AL 35294, USA
2 Department of Obstetrics and Gynecology-Gynecologic Oncology, University of Alabama at Birmingham, Birmingham, AL 35233, USA
3 Division of Pharmacology, Chemistry and Biochemistry, College of Medicine, University of Arizona, Tucson, AZ 85721, USA
4 Cancer Research UK Beatson Institute and Institute of Cancer Sciences, University of Glasgow, Glasgow, UK
transcription factors (TFs) that repress the epithelial phenotype act either directly (SNAI1, SNAI2, SNAI3/SMUC, ZEB1 and ZEB2) or indirectly (TWIST1, TWIST2 and E2.2, GSC, SIX1, and FOXC2) on E-box consensus sequences of E-cadherin’s promoter to repress E-cadherin expression [9–11] which has demonstrated itself to be a key event in EMT. As such, transcriptional control of EMT has been a subject of intense focus revealing multiple redundant, overlapping and tissue specific distinct roles for the TFs’ in this process. Most evident are studies on TWIST and ZEB1 that have different roles in breast versus pancreatic cancers [12–16]. It is also increasingly apparent that EMT regulation is facilitated at multiple non-transcriptional levels including epigenetic, post-translational modifications of the TFs and their associated proteins, and via non-coding RNAs. In recent years, indepth examination of the EMT-TFs and their analysis in vivo and in cancer patients has helped resolve some of the earlier controversies around EMT’s role in metastatic dissemination even in cancer cells or clusters of cells that lack so called EMT hallmark morphological differences [17, 18]. EMT-TF expression, and their activities themselves are regulated by extracellular stimuli, including but not limited to growth factors such as TGFβ and stressors such as inflammation and hypoxia [19, 20]. Such stimuli are tightly coupled to the specific tumor microenvironment (TME). Given the array of cellular and non-cellular components in each TME, unifying principles are challenging to develop and can only emerge upon a complete understanding of all TME components and their roles in a cancer specific manner. Much has been written about EMT and its relationship to TGFβ in cancer metastasis in the past decade [20–22], however in light of significant emerging knowledge on the cellular and acellular factors in the unique cancer microenvironments, new analysis is warranted. Towards this end herein, we focus on the role of EMT and TGFβ in the unique metastatic environment of ovarian cancers. Ovarian cancer follows a metastatic trajectory quite distinct from most other cancers. Patients continue to suffer from a lack of effective targeted therapies, despite the surge in EMT and TGFβ based therapeutic approaches for multiple tumor types.

### TGFβ family in ovarian and related cancers

#### TGFβ superfamily

The discovery of the TGFβs’ can be traced as far back as 1976 when De Larco and Todaro first published in *Nature*, the presence of a group of proteins isolated from conditioned media of malignant tumors, that can induce morphological transformation in fibroblasts and promote growth of cells in soft agar. These proteins were called Transforming Growth Factor (TGF) now widely known as the TGFβ family [23]. Later, three isoforms of TGFβ (TGFβ1, TGFβ2, and TGFβ3) were characterized from this mixture [24–29] and were found to be encoded from individual genes located on different chromosomes [30, 31]. The TGFβ superfamily is a large and expanding group of regulatory polypeptides. TGFβ1 is the archetype [32] with over 50 new members including TGFβ1-3, bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs), activins/inhibins (INHBA-E,INHA), and glial-derived neurotrophic factors (GDNFs), as well as Müllerian inhibiting substance (MIS), also referred to as anti-Müllerian hormone (AMH), nodal growth differentiation factor (Nodal), and left–right determination factor (Lefty) [33, 34] (Table 1). The TGFβ family members exist as either homo- or heterodimeric polypeptides, sharing a conserved cysteine knot structure [35]. All TGFβ isoforms are produced as a latent complex with an N-terminal latency-associated peptide (LAP) and secreted efficiently through interactions with latent TGFβ binding protein (LTBP) [36]. These complexes are rapidly stored in the ECM. Activation can then occur either by proteolytic removal of LAP or via cell-ECM generated forces by integrins (αβδ or αβ8) interacting directly with conserved RGD motifs on the LAP of TGFβ1 and TGFβ3 [37–41] or indirectly via thrombospondin1 [42] and glycoprotein-A repetitions predominant (GARP) in platelets and regulatory T cells (Treg) [43]. Mature TGFβ dimers can then bind receptors to elicit signaling [44]. However not all TGFβs are created the same in terms of physiological functions and in pathologies. TGFβ1 knockout mice succumb to massive weight loss and death at three to five weeks of age due to uncontrolled systemic inflammation, causing lethal immune system defects and multiple inflammatory lesions [45]. Changes in endothelial cell differentiation and hematopoiesis was also reported in the yolk sac of TGFβ1 null mice [46]. The majority of TGFβ2 knockout mice also die before or shortly after birth due to developmental craniofacial defects and defects in the heart, lung, ear, eye, genitourinary tract, and skeleton. TGFβ3 null mice exhibit cleft palate and pulmonary developmental delays that result in early death [47, 48]. Although cleft palate and lung developmental delays were reported in both TGFβ2 and TGFβ3 null mice, the underlying mechanisms were shown to be different [49] with TGFβ3 specifically inducing EMT in the palate [50]. Evidence of cooperativity between the ligands also exists, as binding of TGFβ2 to its receptors is facilitated by TGFβ3 during certain physiological processes [51, 52] and in some contexts, TGFβ1 can cooperate with TGFβ2 to induce EMT [53].

Immunohistological and in situ hybridization studies show overlapping spatiotemporal expression of all TGFβ isoforms in mouse embryos in cartilage, bone, muscle, heart, kidney, ear, eye, blood vessels, lungs, gastrointestinal tract, central nervous system, liver, and skin. No TGFβ3 protein
expression was found in adrenal glands [54] indicating tissue specific distribution of the different isoforms of TGFβ. In the blood vessels, TGFβ1/2 were observed only in smooth muscles while higher levels of TGFβ3 are present in both smooth muscles and the endothelium [54, 55]. In the ovary, TGFβ2 and 3 are present during embryonic development [56] with TGFβ3 mRNA highly expressed in the ovarian surface epithelium (OSE) indicative of its possible pivotal role in the pathophysiology of the OSE [57]. Additionally, ovaries express TGFβ2 in the surface epithelium as well as in the stroma [58]. Differential mRNA expression of all three isoforms were found in fallopian tube epithelial cells with TGFβ1 mostly detected in epithelial cells and TGFβ2/3 equally expressed in both epithelial and non-epithelial cells [59]. TGFβ1 and 2 have demonstrated roles in ovarian cancers, however, it is not clear if TGFβ3 plays a direct role in EOC despite a few lines of evidence indicating potential roles in ovarian angiogenesis [60]. While other members including BMPs, Activins and Inhibins’ have important physiological and pathological roles in the ovary and in ovarian cancer [61–65], here, we focus solely on the TGFβ isoforms.

Signal transduction mechanisms of TGFβ

Signaling and response to TGFβ’s is regulated at multiple levels, including ligand synthesis and activation [66, 67], presence of agonists and antagonists [68, 69] and cell surface receptors and co-receptors expression [44, 70]. The mature forms of TGFβ1-3 have 97% similarity and hence, exhibit redundancy in binding receptors [71, 72]. However, conformational differences exist between TGFβ3 and TGFβ1/2. TGFβ3 is structurally either open or exists as a mixture of closed and open conformations, which leads to more flexible ligand receptor interactions, while TGFβ1 and TGFβ2 are found largely in closed conformations. These differences are thought to play a role in differential biological functions of TGFβ3 as they may impact signal duration and amplitude, a key component in determining TGFβ signaling outcomes [73, 74].

The receptors and SMAD dependent transcriptional control

Cellular response to TGFβ occurs via an extensively characterized and defined cascade of events that are initiated upon binding of ligand to specific receptors [44, 75]. The TGFβ family kinase receptors are all single-pass membrane spanning serine-threonine kinase receptors, consisting of two subfamilies: Type I and Type II receptors (summarized in Table 1).
in Table 1). Despite the large number of TGFβ superfamily members, the number of receptors is limited to five type II and seven type I (ALK1-7) receptors that are essential for signaling but can also exist in multiple heteromeric combinations [62, 75, 76]. An additional class of receptors are the co-receptors/Type III receptors (endoglin and betaglycan) that also bind ligand but act as either ligand reservoirs, modulate intracellular trafficking of internalized ligand and receptor, or increase affinity of the ligand for the serine threonine kinases [70] implicating them as critical for fine tuning signaling responses. Betaglycan and endoglin act as coreceptors for all three isofoms of TGFβ although betaglycan shows higher affinity to TGFβ2 and endoglin to TGFβ1 and 3 [77–79]. Within the heteromeric receptor complexes, the type II receptors phosphorylate and activate the type I receptors [80]. This activation initiates signaling via the

Fig. 1 TGFβ signaling pathways in EMT. Cleavage of the pro-domain latency-associated peptide (LAP) releases active TGFβ that can bind cell surface receptors. Cell surface receptors include the Type III receptor (TGFβRIII/betaglycan), Type II receptor (TGFβRII) and the Type I receptor (TGFβRI) (also see Table 1). TGFβ elicits cellular responses by forming ligand-receptor ternary complexes. Constitutively active TGFβRII transphosphorylates TGFβRI on Ser-thr residues, activating its kinase activity, which in turn phosphorylates SMAD2/3 forms heterocomplexes (heterotrimic or dimeric) with SMAD4 and accumulates in the nucleus to regulate expression of genes associated with EMT. SMAD7 (red) terminates signaling by increasing turnover of the kinase receptors. TGFβ also mediates cellular responses via alternate signaling pathways including (from L-R) TAK1 activation by TGFβRI mediated TRAF6 ubiquitination that can induce NF-κB, JNK, p38MAP kinase signaling; induction of PI3K and AKT-mTOR signaling; TGFβ also regulates the WNT/β-catenin pathway via AKT inhibition of GSK-3β, releasing β-catenin for nuclear accumulation; TGFβ induces activation of RhoA-ROCK signaling; activates MEK/ERK pathway via phosphorylation of ShcA by TGFβRI leading to Ras activation and downstream MAP Kinases; TGFβ promotes interaction between CDC42/RAC1 and PAK2. Activation of TGFβ signaling either via SMAD or alternate pathways can induce expression of several EMT-TFs such as TWIST, SNAIL, ZEB to promote EMT and lead to repression of E-Cadherin. miRNAs and lncRNAs also play a role in TGFβ mediated EMT by either inhibiting or stimulating EMT. miR-34a, -324-5p antagonizes TGFβ-SMAD induction of EMT whereas miR-155, -9, -10b, -181a activate EMT
SMAD-dependent pathways with the phosphorylated GS-domain on TGFβRI serving as a docking site for the receptor-regulated SMAD proteins (R-SMADs), allowing the specific recognition and phosphorylation of the R-SMADs at the SSXS motif in their carboxyl-termini (Fig. 1). The SMAD proteins can be classified as R-SMADs (SMAD1, 2, 3, 5, 8, 9); common-partner SMAD (SMAD4), and the inhibitory SMADs (SMAD6 and 7). The inhibitory SMADs antagonize intracellular signaling through interactions with the activated receptors and R-SMADs and increasing their degradation (Fig. 1). SMAD6 preferentially inhibits BMP signaling [81] while SMAD7 inhibits both the TGFβ and BMP signaling axes [82].

The type I receptor mediates signaling into either of two distinct R-SMAD pathways: TGF-β-SMAD pathway utilizes SMAD2/3 while the BMP-SMAD pathway utilizes SMAD1/5/8 [83]. However significant recent evidence [76, 84, 85] indicates that these SMADs are not exclusive to TGFβ or BMP respectively, adding to the complexity of responses. Phosphorylated complexes of SMAD2/3 or SMAD1/5/8 form a higher-order complex with the co-SMAD4 which then accumulates in the nucleus and binds to regions on the DNA to control transcription of several target genes (Fig. 1). The identification of the membrane receptors and SMAD proteins and analyses of the signaling kinetics in detail [86] have revealed that the diverse cellular responses generated by TGFβ in cells, do not necessarily connote the use of different signaling pathways, but rather, the different interpretation of outputs from the same signaling pathway.

Both the R-SMADs and the co-SMAD (SMAD4) have two conserved Mad homology domains (MH1 and MH2) at the amino and carboxyl terminus respectively [87] separated by a linker region. All R-SMADs except for SMAD2 can bind directly to DNA, via the MH1 domain, although their affinity for DNA is relatively low ($K_d \approx 1 \times 10^{-7} \text{ M}$) [87] compared to other sequence specific transcription factors. SMADs bind to short sequences (SMAD-binding element SBEs) [87] and it is worth stating that a single SBE is not sufficient to recruit an activated SMAD complex. Due to this weak affinity of SMADs to the DNA, specificity of recruitment to DNA usually requires additional protein binding interacting partners via the MH2 domain including co-activators and co-repressors to drive the activation/repression transcriptional program [86, 88] thereby contributing to the contextual responses to TGFβs [89]. Both in the cytoplasm and in the nucleus, the SMAD proteins also undergo additional phosphorylation events in their linker regions that enables peak transcriptional activity including the phosphorylation by cyclin-dependent kinase 8/9 (CDK8/9) a component of the RNA POLII mediator complex and glycogen synthase kinase-3 (GSK3), resulting in the recruitment of YAP and PIN1 respectively to promote transcription and SMAD turnover [90–92]. Notably, CDK8 and YAP1 are required for EMT responses and TGFβ dependent metastasis in multiple models [93, 94]. Thus, TGFβ signals are interpreted in different ways resulting in the diverse responses depending on the cellular context, particularly relevant to the pathophysiology of cancer and EMT. Much has also been discussed about TGFβ and its co-operation with other signaling pathways including the Ras-MAPK and Wnt pathways in promoting EMT by increasing expression of the transcriptional repressors of E-cadherin TWIST, SNAIL and ZEB1 [20] (Fig. 1). Co-repressor complexes of SNAIL1 and SMAD4 can co-silence several tight junction proteins during EMT demonstrating SMAD dependency in EMT induction by TGFβ [95]. Much of the current research focus is on each individual TGFβ member, but the interplay between the different members in cancer and EMT remains to be fully elucidated.

**SMAD dependent non-transcriptional mechanisms**

Transcriptional control is the most investigated outcome of SMAD activation downstream of TGFβ, however significant SMAD functions in the cytoplasm have emerged particularly in RNA processing. SMADs have been implicated in RNA splicing, micro-RNA processing, as well as directly in miRNA mediated EMT (Fig. 1). For instance, miR-23a targets E-cadherin through the TGFβ/SMAD pathway to promote EMT [96]. Other miRNAs such as miR-155 [97], miR-9 [98] and miR10b [99] can also promote EMT via direct targeting of E-cadherin mRNA [100]. There are also numerous inhibitory miRNAs that counter the effect of TGFβ/1/2 on EMT; for example, miR-34a inhibits SMAD4 [101] and miR-324-5p suppresses TGFβ2 dependent EMT [102]. In ovarian cancer, the effect of a subset of miRNAs on EMT has also been well demonstrated as miR-181a promotes TGFβ mediated EMT via the repression of SMAD7 [103]. In contrast, miR-200s are highly expressed in ovarian cancer and correlate with an epithelial phenotype acting to inhibit EMT by targeting SMAD2 and SMAD3 [104]. A few additional mechanisms include roles for RNA binding proteins such as hnRNP E1(heterogeneous nuclear ribonucleoproteins) involved in mRNA processing events wherein, binding of a structural 33-nucleotide TGFβ-activated translation (BAT) element in the 3’Untranslated region of disabled-2 (Dab2) and interleukin-like EMT inducer (ILEI) transcript in response to TGFβ signaling promotes EMT [105].

Long non-coding RNAs (lncRNA) have also emerged as key regulators of TGFβ mediated EMT (Fig. 1) including H19 [106, 107], LINK-A [108] DNM3OS [109] MALAT1 [110, 111], PVT1 [112] PE [113], and BORG [114–116]. Many act as sponges for miRNAs as in the case of H19, MALAT1, LncRNA-ATB and LncRNA PTAR that sponge miR-370-3p, miR-30a, miR-200 and miR-101-3p respectively to enhance either ZEB1/2 or TWIST1 expression
during EMT or during EMT mediated metastatic outgrowth as in the case of BORG [110, 114–118]. MALAT1, which is frequently upregulated in EOC, also partakes in EMT by interacting with EZH2 and by recruiting chromatin modifiers [119–121] and induces formation of an lncRNA-protein complex containing Smads, SETD2 and PPM1A phosphatase leading to dephosphorylation of TGFβ/Smad signaling [111]. Lastly, an under investigated area of regulation of EMT by TGFβ in the cytoplasm is SMAD’s potential role in mitochondrial function [122] that has emerged as an important player in regulating ovarian cancer metastasis [123]. Specifically, SMAD2 interacts with mitofusin2 (MFN2) and Rab and Ras Interactor 1 (RIN1) to promote mitochondrial fusion [122]. Whether these specific interactions result in mitochondrial and/or metabolic alterations and EMT however remain to be tested.

**SMAD independent pathways**

TGFβs can also activate a series of non-SMAD signaling pathways with similar and/or delayed kinetics to the SMAD pathways in a context dependent manner [124]. Most commonly, these pathways are activated directly by the Type II and Type I receptors or through the Type III co- receptors [124–127]. In the context of EMT, mitogen-activated protein kinase (MAPK) pathways Jun-N terminal kinase (JNK), extracellular-signal-regulated kinases 1/2 (ERK1/ERK2), p38 and PI3K kinases; AKT/PKB pathway, small GTP-binding proteins, RhoA, Rac1 and CDC42, and mTOR; protein tyrosine kinases such as PTK2, SRC and ABL, and the NF-κB pathway and Wnt/β-catenin signaling pathway have been examined in TGFβ dependent EMT and are reviewed elsewhere in detail and are summarized in Fig. 1 [128–130]. An example is the activation of ERK MAP kinase by TGFβ1 via the phosphorylation of the scaffold protein ShcA by the Type I receptor ALK5 [131] or phosphorylation of the Type II receptor directly by Src [132] leading to Ras and MAPK activation [133]. Consistently, inhibition of ERK MAP kinases inhibits TGFβ induced EMT [134]. Other mechanisms include ubiquitination mechanisms that also depend on Type I receptor interactions with tumor necrosis factor receptor-associated factor 6 (TRAF6) and the subsequent activation of the MAPKKK TAK1 upstream activator of JNK and p38 [135, 136] which is required for TGFβ-induced EMT, in non-ovarian cancers [128, 137]. TGFβ2 has also been shown to utilize both SMAD and non SMAD mechanisms in a subset of cancers to promote EMT and invasion via autophagic responses [138, 139]. Much like the Type I receptor, the Type II receptor TGFβRII can also phosphorylate other proteins besides the TGFβ receptors to impact EMT. At the level of cell- cell junctions, TGFβ regulates RhoA activity through Par6 interactions with TGFβRI leading to TGFβRII mediated phosphorylation of Par6 [140] and subsequent RhoA degradation at tight junctions [140] (Fig. 1). Thus, the TGFβ serine threonine kinases can have substrates beyond the SMADs and TGFβ receptors and vice versa the receptors can be phosphorylated by a variety of kinases, that may be relevant during EMT and other process. Given that the Type II receptors of the TGFβ family have been identified in genomic studies as driver protein kinases in about 5–15% of cancers [141], identification of all their substrates will likely shed light on additional mechanisms including EMT in cancer.

**TGFβ alterations and sources in epithelial ovarian cancer**

Mutation hotspots exist in genes that encode a subset of TGFβ ligands and receptors (TGFβR2, AVCR2A, BMPR2), and SMADs (SMAD2, SMAD4) in many non-gynecological cancers [142]. In high grade serous EOCs’ (HGS), amplification frequency of the TGFβ pathway components listed above was found to be high, consistent with high genomic instability of these EOCs [142–144]. In the fallopian tube which is one of the sites of tumor initiation and early metastasis of HGS cancers, all three TGFβ isoforms and their receptors are expressed, with most reports indicating elevation of all three isoforms in primary, metastatic and recurrent EOCs compared to normal ovaries [58, 59]. However, a clear prognostic value for these changes has only recently emerged with increased access to genomic data, publicly available data sets and tools for investigators to analyze these including TCGA, KMplotter, Oncomine and DepMap to indicate a few. Such studies have revealed lack of a robust correlation between TGFβ1 expression and survival outcomes in women with ovarian cancer. However most notably, increased TGFβ2 and TGFβ3 mRNA expression were associated with poorer prognosis based on worse progression-free survival (PFS) and reduced overall survival (OS) [145]. While the utility of the TGFβ ligand expression as a biomarker continues to be debated, there is significant evidence that all isoforms are produced albeit at different locations and to different degrees. Indeed the source of TGFβ in ovarian cancers has been reported to be not just the tumor cells, but also the peritoneal mesothelium and tumor infiltrating cells [146]. Thus, understanding the specialized local sources and mechanisms of latent TGFβ activation during metastasis is likely more relevant to delineating specific TGFβ dependent outcomes in ovarian cancer.
EMT and metastasis in epithelial ovarian cancer

Ovarian cancer subtypes and metastatic route

Ovarian cancer remains one of the leading causes of cancer-related deaths in women accounting for 4.4% mortality worldwide [147] in part due to inadequate prevention and detection methods, and ineffective and insufficient therapies for advanced stage patients (Stage III or Stage IV). Ovarian cancers can be classified based on their cell of origin as either epithelial, germ or stromal type [148]. Epithelial tumors are more common in the population and include low and high grade serous (HGS), endometrial, clear cell and mucinous subtypes. Within the epithelial tumors, significant genomic heterogeneity exists and an in-depth understanding of the differences between the subtypes is in fact required to improve precision medicine for these cancers [149]. The most common and aggressive subtype are the HGS, marked by a p53 mutational signature, early genetic instability and genomic heterogeneity [150, 151]. Gene expression profiling and subsequent clustering of these HGS cancers has led to the establishment of additional molecular subclasses that have been evaluated by TCGA as well [152]. In accordance with gene expression signatures, specific clusters were identified and divided into mesenchymal, immunoreactive, differentiated, and proliferative. In comparing survival outcomes, the immunoreactive subclass showed the best survival outcomes among all [153]. Additional classifications have also been proposed based on lesion size and spread in the peritoneum [154]. Notably, comparing EMT gene signatures revealed that peritoneal spread made up primarily of bigger implants correlated significantly with a reduced epithelial status as compared to widespread smaller lesions [154]. Of all patients diagnosed with serous ovarian carcinoma, ~15% have germline BRCA mutations [155]. Although BRCA1 and BRCA2 are intimately involved in DNA damage repair, direct links to TGFβ related EMT in ovarian cancer are emerging. A recent study in ovarian cancer reported that loss of endogenous BRCA1 dampens the tumor suppressive/growth inhibitory effect of TGFβ [156]. Several studies in breast cancer have established links through either BRCA1 dependent transcriptional regulation of EMT transcription factors, cytoskeletal proteins or micro RNAs which may indirectly support TGFβ dependent EMT [97]. Whether these mechanisms are active in ovarian cancers is currently unknown. Identifying the precise site of origin (ovary versus fallopian tube) and mapping the discrete metastatic steps in ovarian cancer has been challenging as compared to other cancer types. However, significant genetic and whole exome sequencing data point to the involvement of p53 mutated serous tubal intraepithelial carcinomas (STIC) lesions in the fallopian tube early on, with subsequent or continued metastasis into the fallopian tube epithelium, ovaries, peritoneum, omentum, uterus, pelvic walls and occasionally to the rectum and bladder [157–160] (Fig. 2). EOC metastasis is thus largely transcoelomic, with some evidence of hematogenous and lymphatic spread [161, 162] (Fig. 2). However, the contributions of hematogenous and lymphatic spread of EOC metastasis remain limited and somewhat controversial.

Role of EMT in initiation of HGS EOC metastasis

EMT is part of the normal ovarian physiology during oocyte release [163]. Thus, it is likely that EOC tumor cells retain the capacity to transform and convert into a more mesenchymal state naturally and thereby invade into the peritoneum. One accepted model of peritoneal spread involves the detachment and shedding of cells from the tumor, which would likely require weakening of some cell–cell junctions and cell–ECM interactions (a hallmark of EMT) followed by survival under anchorage independence, re-attachment to new locations and establishment of new colonies within the transcoelomic/intraperitoneal cavity (Fig. 2). The detachment/shedding can be as single cells or clusters of cells as both are detected in the ascites of advanced EOC patients [164] (Fig. 2). Both cell survival and invasion events are associated with a mesenchymal gene and protein expression profile as cells that are able to grow under anchorage independence (a critical step during metastasis) exhibit a more mesenchymal phenotype, expressing high N-cadherin and low E-cadherin [165, 166]. Whether EMT is a driver for initiating shedding is somewhat unclear. Immunohistological analysis of both primary and metastatic ovarian carcinoma however reveal that EMT is significantly associated with peritoneal metastasis and correlated with low survival outcomes for ovarian cancer patients [167, 168]. Most recently, miR-181a, that can promote EMT by inhibiting SMAD7 (Fig. 1) has been reported to promote oncogenic transformation by increasing genomic instability in fallopian tube epithelial cells, in part through effects on the tumor suppressive timulator of interferon genes (STING) pathway [169]. STING and genome instability have in other cancers been reported to be associated with a mesenchymal signature and metastasis, wherein cells with high chromosome instability were enriched for EMT associated genes and pathways [170]. Thus, it is likely that the convergence of STING, chromosome instability and EMT factors, contribute both to cellular transformation and initiation of metastasis in ovarian cancers (Fig. 2). Here again, the precise source of TGFβ within the tumor in the fallopian tube or ovary, that may directly facilitate EMT needs to be defined as it
may vary depending on the type of tumor, e.g., immunoreactive, versus mesenchymal or proliferative versus differentiated. Several lines of evidence indicate that within the tumor, local TGFβ secretion from infiltrating stromal cells, leukocytes, macrophages, and myeloid precursor cells could create a more favorable proinflammatory microenvironment for EMT [171]. For instance, release of active TGFβ via GARP produced from tolerogenic Treg cells has been shown to promote EMT and immune tolerance [172]. Similarly, TNF-α, which might be a result of infiltrating monocytes has been shown to promote EMT sensitivity as well [173]. A second environmental factor that could create an EMT conducive tumor is hypoxia, which either via HIF1/2, NFκB and/or changes in the redox environment has the potential
The peritoneum is a membrane of mesothelial cells, which lines the wall of the abdominal cavity, lying on abdominal and pelvic organs, including the omentum. The peritoneal mesothelial cells (PMCs) create a mechanical barrier for the abdominal organs. The peritoneal cavity acts as a rich “soil” of ECM proteins such as collagen I and other adhesion molecules that can support cell proliferation, migration, and invasion. The peritoneum thus provides a site for EOC cells, aggregates, and clusters to attach and invade [178–180]. In this environment tumor cells have been shown to interact with the PMCs via TGFβ signaling, wherein cancer cell derived TGFβ1, via TGFβ1 receptor interactions on the PMCs’ activates a RAC1/SMAD pathway, leading to increased fibronectin expression and a mesenchymal phenotype in the PMCs [181]. Such studies establish the mesothelium as an active player in metastasis, with TGFβ and the mechanisms of EMT central to the invasion process. In this scenario, it is possible that cells, prior to attachment exist in mesenchymal or partial mesenchymal states (Fig. 2). These states could be acquired as a result of EMT during detachment from the primary tumor sites (Fig. 2) or acquired while under anchorage independence as a result of inter-cellular interactions (Fig. 3), thereby priming the cells for a complete EMT transition and effective peritoneal invasion. Kinetic models of EMT that incorporate multiple states of EMT [182] will be required to address this in detail in the future.

A variation on the classic cancer EMT of tumor cells is mesothelial-mesenchymal transition (MMT) of the PMCs, wherein PMCs transition into mesenchymal cells (MCs), acquiring migratory and fibroblast-like phenotypes [183], much like cancer associated fibroblast (CAFs). MMT has been extensively investigated in other tissues during fibrosis of the peritoneal membrane and in cancers such as mesothelioma [184]. The “activation”/MMT process could thus play an active role in facilitating metastasis as TGFβ is also produced by the peritoneum itself [185]. Partnering with EOC cells to invade the mesothelium are CAFs. CAFs have been shown to mediate invasion through the sub-mesothelial layer by producing ECM components and growth factors turning the peritoneal cavity into a metastasis conducive niche for EOC cell attachment and metastasis [186]. Additional mechanisms of peritoneal invasion utilize integrins. Of note CAFs in the ascites of EOC patients are present in aggregates with tumor cell clusters that express integrin α5 and are highly efficient as a unit at invading and metastasizing into the peritoneum as a result of TGFβ signaling activation [187]. Increased expression of β1-integrin has also been shown to result in direct cell–cell interactions between EOCs and PMCs as confirmed by electron microscopy and adhesion studies [186]. Thus, while several studies have demonstrated the role of mechanical ‘pushing’ forces where cell clusters in the form of spheroids can impose integrin and myosin generated forces to invade the mesothelial layer, destruction of the mesothelium may be a prerequisite for invasion. Indeed, tumor induced apoptosis of the mesothelium via the Fas/FasL pathway has been shown to promote PMC clearance and invasion at the initial stages of metastasis [188, 189]. Whether TGFβ plays a direct role in dictating both EMT and apoptosis in the mesothelium is worth speculating, as TGFβ can in fact concurrently induce both apoptosis and EMT as demonstrated in pancreatic and liver cancers [190, 191] and perhaps in other systems as well. Lastly, but far from the least, the role of the immune environment in peritoneal spread of EOCs is critical as depending on the number and size of peritoneal lesions, the peritoneal microenvironment...
can present either a more adaptive response or a more systemic inflammatory response [192].

The ascites

As EOC progresses, metastatic fluid (ascites) accumulates in the peritoneum. Increasing volumes of ascites are thought to generate a favorable tumor microenvironment, enabling transcoelomic tumor spread in a feed forward manner. As such ascitic volume and components have been used to grade, predict stage and survival outcomes including chemoresistance outcomes of EOC patients. Some studies also suggest ascites to be an independent prognostic factor in EOC [193–195]. Ascites can also contribute to morbidity due to gastrointestinal problems and while in most cases treating the underlying disease will reduce ascites, untreatable ascites can be a recurrent and frequent problem requiring drainage and paracentesis. Ascites accumulation in EOCs has been attributed to multiple factors including increased vascular permeability of vessels lining the peritoneum, lymphatic obstruction leading to reduced lymph drainage and also angiogenesis triggered by tumor cells and CAFs attached to the peritoneal wall [196, 197]. In this context, TGFβ blockade prevents destruction of the lymphatic vessels leading to control of ascites acting in part through VEGF inhibition [198]. TGFβ1 that has been reported to be elevated in the ascites of EOC patients [199, 200]. In-depth analysis of the other TGFβ isoforms is currently lacking and...
this may further shed light on TGFβ2/3’s role as well in the future.

The ascites constitutes its own environmental niche (Fig. 3) as it not only constitutes much of the same cellular components from the primary tumors such as the tumor cells, immune cells, fibroblasts, endothelial cells and lymphocytes but also harbors mesothelial cells [201] and likely tumor cells shed from metastatic sites as a result of forces from the ascites current (Figs. 2, 3). In general, approximately 37% of the cellular composition of the ascites constitutes lymphocytes. In general, approximately 37% of the cellular composition of the ascites constitutes lymphocytes, about 30% macrophages and mesothelial cells and 0.1–0.5% constitutes carcinoma cells [201] and the remaining includes fibroblasts and endothelial cells. A recent single cell analysis that examined EMT signatures of single and aggregate tumor cells from the ascites not only confirmed the heterogeneous mix of the ascites, but also demonstrated a strong EMT program that was dependent on the CAFs and notably TGFβ [202]. These findings are also consistent with prior reports demonstrating the impact of the ascites on inducing EMT in cell line models [203]. Nevertheless, not just would EMT of the cells in the ascites promote peritoneal invasion and metastasis, but also contribute to anoikis resistance. While much has been debated on whether cells under anchorage independence in the ascites are truly matrix detached, survival in the ascites requires all the cells in the environment to have adapted to changes in matrix attachment. Nonetheless, anoikis resistance is an accepted pre-requisite of malignancy [204] and has been strongly linked to an EMT signature [166].

In addition to the heterogeneous cellular population in the ascites that can produce and respond to TGFβ, several recent studies have also characterized the presence of exosomes (Fig. 3) that have been proposed as biomarkers in EOC as studies have also characterized the presence of exosomes in ascites that can produce and respond to TGFβ, several recent studies have strongly linked to an EMT signature [166]. The omentum is a specialized adipose tissue in the peritoneal cavity and is also the most preferred metastatic site for HGS cancers [212]. Acquisition of EMT characteristics has been shown to depend on cells in the omentum in mouse models [213]. However, in clinical practice, performance of omentectomies in patients without bulky disease or a grossly normal omentum has not yet been definitively shown to improve survival [214]. The omental microenvironment is undoubtedly highly conducive for tumor growth via both metabolic and immunological factors as evidenced by advanced EOC patients exhibiting significant omental tumor load. The omentum has also emerged as a pre-metastatic niche for progression and development of invasive HGS cancers [215]. The omentum is a highly vascularized tissue and contains ‘milky spots’ (in both humans and mice) which are primarily aggregates of leukocytes referred to as fat-associated lymphoid clusters (FALCs) [216] (Fig. 3). The lymphatics of the omentum serve as a conduit for fluid drainage from the peritoneum making it an ideal spot for tumor cells to land. Recent studies have used 3D coculture models indicating that EOC tumor cells via TGFβ can stimulate activation and proliferation of omental resident fibroblasts that in turn stimulates cancer cell adhesion, invasion and peritoneal dissemination [217]. In a corollary fashion, a ten gene signature that includes collagen-remodeling genes regulated by TGFβ1 signaling has been correlated with increased metastasis and poor patient survival [218]. This is particularly significant as the omentum is collagen rich and serves as a robust site for tumor cell adhesion via integrins [219].

Analogous to the peritoneum, mesothelial cells in the omentum can also secrete TGFβ that impacts the fibroblasts and tumor cells, and also the immune state of the omentum [220]. The omentum also hosts a unique macrophage population, expressing CD163 and Tim4 that can interact with EOC cells to promote metastasis [213]. Tumor Associated macrophages (TAMs) are a significant source of TGFβ and other EMT mediators [221–223] playing key roles in creating an immune suppressive environment in the omentum [215, 220]. Thus several recent studies have focused on understanding the immune microenvironment of the milky spots in the omentum [220]. Adipocytes are the other cell type highly enriched in the omentum that have seen a symbiotic relationship with EOCs, and are coined cancer associated adipocytes (CAA) [224] (Fig. 3). These CAs can act as powerhouse during advanced disease, providing the necessary energy for rapidly growing tumor cells via FABP4 [212], a chaperone for free fatty acids. FABP4 levels are indicators of increased residual disease after primary debulking surgery of advanced HGS patients [225]. In some in vitro
systems FABP4 has been shown to promote EMT via TGFβ [226]. Other CAA derived chemokines have been shown to support tumor progression by inducing a partial EMT in breast cancer models [227] and can cooperate with endotrophin, a cleavage product of collagen VI α3 chain to promote EMT [228]. Worth noting is the role of TGFβ as a strong negative regulator of adipogenesis, acting via non-SMAD mechanisms in breast cancer [229]. In the same study [229] adipogenesis induction reduced invasiveness with the CAAs localized to tumor borders [229]. The interplay between adipogenesis and EMT in the omentum of metastasized HGS ovarian cancer patients remains to be determined.

Targeting TGFβ and EMT for EOC management

EOC specific challenges

Complete debulking surgery is commonly the first-line of treatment for EOCs, followed up with a carboplatin and paclitaxel chemotherapy regimen. Most EOC patient tumors fall under one of two categories, either they are chemo-resistant at the outset, or will eventually become chemo-resistant. Thus, management of recurrent and resistant disease is one of the biggest challenges for EOC. Approximately 50% of EOC patients who have alterations in BRCA1/2, and/or alterations in other homologous recombination repair deficient pathways (HRD genes), are more likely to be carboplatin sensitive. For such patients, PARP inhibitors hold great promise with significant increases in PFS reported [230]. However, there is a dearth of treatment options for the remaining 50% of patients. Most of these patients respond well initially, but more than 70–80% of patients overall will relapse in less than 5 years [231] regardless of the original response status. Thus, disease management to improve and prolong survival remains a continuous challenge.

Chemo-resistant tumors express a more mesenchymal gene signature that also coincides with stem cell like features [232]. Notably, at the completion of primary platinum-based chemotherapy, HGS ovarian cancer patients were found to express high levels of cancer stem cell (CSCs) markers such as CD44 (a non-kinase transmembrane glycoprotein), CD133 or prominin-1, Aldehyde Dehydrogenase 1 Family Member A1 (ALDH1A1) as compared to primary tumors. Thus CSC enrichment during courses of chemotherapy may have contributed to resistance and relapse [233]. Several molecular mechanisms have been proposed for resistance acquisition by CSCs such as autophagy for survival by recycling metabolites [234]; high expression of ATP-binding cassette (ABC) transporters to increase drug efflux [235, 236] and increased DNA polymerase η (Pol η) synthesis to compensate for drug induced DNA damage [237]. Several of these mechanisms are in fact downstream of EMT [238] and EMT transcription factors. Specifically, the EMT transcription factor SNAIL has been shown to be required to maintain stem like features in multiple ovarian cancer models [239, 240] in part via tumor suppressor miRNA let-7 [239]. ZEB1 has also been shown to promote EMT and stemness by increasing SOX2, OCT4, NANOG, CD44, and CD117 expression resulting in resistance to cisplatin [241]. Such studies strongly suggest TGFβ induced EMT as a mechanism to promote stemness in ovarian cancers. In breast cancers, increased TGFβ signaling via increased cell surface receptor expression has been directly linked to chemoresistance [242]. These cells can be resensitized to chemotherapy using Galunisertib or LY21567299 (small molecule TGFβR1 inhibitor (Fig. 4 and [242])). Whether this approach is effective in EOCs remains to be determined.

Targeting EMT

Being able to target EMT related pathways has been a landmark development for cancer therapies. Several approaches and therapeutic targets have been developed or are being evaluated [243] including in EOC (Table 2). EMT itself can be targeted at multiple levels including, (a) blocking signals that induce EMT (including TGFβ inhibitors (see section below)), (b) blocking downstream transducers (such as tyrosine kinases and miRNAs) [244] (c) blocking mesenchymal mediators such as fibronectin, vimentin and N-cadherin (such as Artemisinin that reduces vimentin expression and can reverse EMT) [245, 246] and (d) blocking the MET transition which has been shown to be mediated by other members of the TGFβ family particularly BMP7 [247, 248]. Additional approaches include exploiting tumor vulnerabilities that arise as a result of EMT. One example is the exploitation of the plasticity and programmability of cells undergoing EMT as seen in breast cancer models wherein cells were forced to transdifferentiate into adipocytes, which in turn reduced metastasis [249]. An additional dependency that has garnered significant attention is metabolic adaptation to meet increased energy demands during cancer metastasis [250, 251]. One candidate with promise in EOC, is metformin, which in preclinical models can inhibit EMT, stemness and reverse chemotherapy resistance. Most notably, in a recent Phase II study with HGS patients, metformin was found to be well tolerated and reduced the stem cell population in these patient tumors. Improved OS was also observed, prompting Phase III studies for the future [252]. Thus, targeting ovarian cancer with standard of care in
combination with metabolic inhibitors could suppress EMT related progression and yield promising results.

**TGFβ specific targeting strategies and clinical progress**

TGFβ specific targeting can occur at multiple steps (Fig. 4) including (a) at the level of TGFβ ligand activation and directly limiting ligand availability (b) at the level of receptor inhibition and blocking receptor kinase activity (c) inhibition of transcription regulation by SMADs (d) indirectly by immune based strategies. Several antibodies, peptides, small molecule inhibitors and receptor trap-based approaches have been developed many of which are in clinical trials (Fig. 4 and Table 2). However, given the discovery of TGFβs in the late 1970s, progress to the clinic has been relatively slow, in part due to the discovery of cardiac toxicity in dogs after continuous administration of a small molecule inhibitor to TGFβR1 [253] and the complex roles of TGFβ itself in cancer progression acting either as a tumor suppressor or tumor promoter in a context dependent manner. Defining the source and role of TGFβ particularly in the stroma to promote EMT and metastasis, and in the distinct immune cell types, including CD4+ , CD8+T cells, NK or dendritic cells [254], has provided much renewed faith in therapeutic targeting of TGFβ in cancer.

**Ligand control**

TGFβ ligand neutralizing antibodies and antibodies that block TGFβ receptor ligand interactions have received significant pharmaceutical and clinical attention, however with limited progress specifically in ovarian cancers. Fresolimumab ([255]) (Sanofi, GC1008) is a high affinity
fully human monoclonal antibody that is a pan neutralizing antibody for all three TGFβ isoforms with promising clinical findings in non-ovarian cancers and particularly breast cancer patients receiving radiotherapy [255, 256]. Fresolimumab is also being explored for the treatment of Osteogenesis Imperfecta (OI) as animal model studies indicate that silencing TGFβ increases bone mass [257]. The findings from the OI trials could have beneficial side effects for the management of ovarian cancer patients as well who are mostly menopausal and over time suffer significant bone mass loss [258]. Trabedersen (AP12009 or OT-101) has also emerged as an alternative strategy, which is an antisense approach targeting TGFβ specifically and has shown significant promise in pre-clinical ovarian cancer models [259]. Another approach to block TGFβ at the ligand level is to block activation using anti-integrin approaches [260] and anti-GARP (ABBV151) approaches [261]. GARP is specifically required for TGFβ activation in regulatory T cells (Tregs) and platelets and ABBV151 is currently in clinical trials (Table 2) in combination with immune checkpoint inhibitors (ICI). Other approaches in the research and development phases that have or are being explored include the development of a high affinity engineered TGFβ monomer that acts as a dominant negative due to its inability to dimerize with TGFβRII and activate signaling, and a peptide ligand trap based approach that utilizes a sequence of the Type III TGFβ receptor (betaglycan) [262, 263]. The use of betaglycan based approaches is quite attractive as the domains by which it binds the different TGFβ members has been well mapped [264] providing potential for selective inhibition of not just the TGFβ isoforms but also beyond for other TGFβ members including Inhibins, Activins and BMPs [265]. Current TGFβ isoform specific traps include AVID200 that blocks TGFβ1 and TGFβ3 and is currently in clinical trials (Table 2). Thus, delineating isoform specific effects remains important for the long-term success of such agents in EOC and other cancers.

### Receptor activation and transcriptional inhibitors

Small molecule-based approaches (Table 2, Fig. 4) have historically been the favorite approach for targeting TGFβ signaling at the reception level due to their ease of administration.

#### Table 2 Summary of TGFβ targeting drugs in clinical trials

| Name                        | Mechanism of action                        | Cancer type                              | Clinical trial identifier number | Ovarian cancer patient recruitment | Reference |
|------------------------------|-------------------------------------------|------------------------------------------|----------------------------------|-----------------------------------|-----------|
| Galunisertib (LY2157299)     | TGFβRI kinase inhibitor                    | Advanced Hepatocellular carcinoma        | NCT02240433                      | No                                 | [282]     |
| Fresolimumab (GC100800)      | Anti-TGFβ monoclonal antibody              | Advanced malignant melanoma or renal cell carcinoma | NCT00356460                      | No                                 | [283]     |
| TβM1                         | Anti-TGFβ1 monoclonal antibody             | Adenocarcinoma of the colon              | –                                | No                                 | [284]     |
| NIS793                       | Anti-TGFβ antibody                         | Advanced malignancies                    | NCT02947165                      | No                                 | [285]     |
| Trabedersen (AP12009 or OT-101) | Synthetic TGFβ2 antisense oligodeoxynucleotide | Glioblastoma multiforme or Anaplastic astrocytoma | NCT00431561                      | No                                 | [286]     |
| ABBV151                      | GARP binding, which interferes with production and release of active TGF β Tregs | Advanced Solid tumors                    | NCT03821935                      | Yes                                | [287]     |
| STM 434                      | Soluble receptor ligand trap targeting Activin A | Ovarian Cancer( granulosa cell tumors) and other Advanced solid tumors | NCT02262455                      | No                                 | [265]     |
| AVID200                      | TGFβ1 and TGFβ 3 neutralizing antibody     | Malignant Solid Tumors                   | NCT03834662                      | No                                 | [288]     |
| Vactosertib (TEW-7197)       | TGFβRI/ALK5 inhibitor                      | Advanced solid tumors                    | NCT02160106                      | Yes                                | [289]     |
| PE-03446962                  | ALK1 inhibitor                             | Advanced solid tumors                    | NCT00557856                      | Yes                                | [290]     |
| Bintrafusalfa (M7824)        | Bifunctional fusion protein that sequesters TGFβ and blocks PD-L1 | Non-small cell lung cancer, HER2 positive breast cancer | NCT02517398                      | No                                 | [278]     |
| Belagenpumatucel-L (Lucanix) | TGFβ2 antisense modified non-viral based allogenic tumor cell vaccine | Non-small-cell lung cancer at different stages | NCT00676507                      | No                                 | [291]     |
| TAG vaccine                  | Vector co-expressing GM-CSF                | Advanced metastatic                      | NCT00684294                      | Yes                                | [292]     |
Immune options

Mechanisms to re-activate the immune system and improve the efficacy of immune checkpoint inhibitors (ICI) in cancers particularly ovarian cancers, is currently being actively explored as evidence in multiple other cancer studies indicates this may be an effective approach [276].

That the anti-metastatic effects of the TGFβ inhibitors are potentiated by this combination is also strongly based on prior studies that non-responders to checkpoint inhibitors have elevated levels of the central TGFβ pathway components (TGFβ1, Type I and II receptors) [277]. Supporting this, M7824 a bifunctional fusion protein composed of a monoclonal antibody against PD-L1 fused to the extracellular domain of TGFβRII, is preclinically effective at suppressing metastasis and also provided antitumor immunity [278]. M7824 is currently in clinical trials (Table 2) for multiple solid tumors. Preclinical studies using other approaches including the use of LY2109761 in adoptive T-cell therapy have also been proposed as a way to effectively increase immunotherapy efficacy [279]. Other approaches include whole cell vaccine based methods that use autologous tumor cells expressing an anti-sense to TGFβ2 [280] or tumor-specific CD8+ T cells modified to express a dominant-negative TGFβRII (non-ovarian cancers) [279]. Phase I trials of the vaccine approach have shown low toxicity and durable responses so far [281].

Future outlook with TGFβ based therapeutics in ovarian cancers.

Progress through the clinical pipelines for ovarian cancers has been slow with a major gap being lack of biomarkers to stratify patients and identify those who will benefit the most. This is a central issue for the progression of targeted therapies in EOC. With the acceptance that TGFβ inhibitors are likely to be most beneficial in combination therapy as opposed to as a monotherapy, either with immune checkpoint inhibitors or other approaches including anti-angiogenic strategies (not reviewed here), DNA intercalating agents and even with PARP inhibition, the outlook for the expanded use of TGFβ inhibitors in ovarian cancers remains positive.

Concluding remarks

TGFβ signaling mechanisms have been examined for several decades. Yet fundamental insights both into the mechanism of action and the context of action continue to emerge. The recent advances outlined here have revealed the broad impact of TGFβ signaling mechanisms in EMT and cancer. These combined with our growing understanding of unique disease environments (such as discussed here for ovarian cancer) have provided new contextual information and understanding the detailed mechanisms by which plasticity is dictated will be critical for the future. With the advent of several TGFβ approaches in the clinic, such momentum is prescient. Emphasis should also be laid on understanding the interplay between the multiple TGFβ members that have in recent years emerged as playing key roles in cancer progression.

Acknowledgements We thank members of Mythreye Karthikeyan’s laboratory particularly Eduardo Listik and Alex Seok Choi for useful feedback. We also want to acknowledge and apologize to the several investigators whose highly relevant studies could not be cited directly here due to space constraints. Figures were created with BioRender.com

Author contributions The manuscript was written and edited by all authors.

Funding For the work was partially supported by NIH Grants R01CA219495 and R01CA230628 to Mythreye Karthikeyan (KM), and NIH grant R01GM128055 to Nam Y Lee.
Compliance with ethical standards

Conflict of interest The authors declare no competing financial interests or conflicts of interest.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

1. Nisticò P, Bissell MJ, Radisky DC (2012) Epithelial-mesenchymal transition: general principles and pathological relevance with special emphasis on the role of matrix metalloproteinases. Cold Spring Harb Perspect Biol 4(2):a011908
2. Hay ED (1995) An overview of epithlieo-mesenchymal transformation. Cells Tissues Organs 154(1):8–20
3. Micalizzi DS, Farabaugh SM, Ford HL (2010) Epithelial-mesenchymal transition in cancer: parallels between normal development and tumor progression. J Mammary Gland Biol Neoplasia 15(2):117–134
4. Kalluri R, Weinberg RA (2009) The basics of epithelial-mesenchymal transition. J Clin Invest 119(6):1420–1428
5. Jeon H-M, Lee J (2017) MET: roles in epithelial-mesenchymal transition and cancer stemness. Ann Transl Med 5(1):5
6. Aiello NM et al (2018) EMT subtype influences epithelial plasticity and mode of cell migration. Dev Cell 45(6):681–695
7. Pastushenko I, Blanpain C (2019) EMT transition states during tumor progression and metastasis. Trends Cell Biol 29(3):212–226
8. Chin VL, Lim CL (2019) Epithelial-mesenchymal plasticity-engaging stemness in an interplay of phenotypes. Stem Cell Investig 6:25–27
9. Peinado H et al (2004) Snail and E47 repressors of E-cadherin. Nature 428(6985):549–554
10. Zheng X et al (2015) Epithelial-to-mesenchymal transition is dispensable for metastasis but induces chemoresistance in pancreatic cancer. Nature 527(7579):525–530
11. Aco-N N (2020) Bring along your friends: Homotypic and heterotypic circulating tumor cell clustering to accelerate metastasis. Biomed J 43(1):18–23
12. Imani S et al (2016) Prognostic value of EMT-inducing transcription factors (EMT-TFs) in metastatic breast cancer: a systematic review and meta-analysis. Sci Rep 6:28587
13. Lopez-Novoa JM, Nieto MA (2009) Inflammation and EMT: an alliance towards organ fibrosis and cancer progression. EMBO Mol Med 1(6–7):303–314
14. Lamouille S, Xu J, Derynck R (2014) Molecular mechanisms of epithelial-mesenchymal transition. Nat Rev Mol Cell Biol 15(3):178–196
15. Morrison CD, Parvani JG, Schiemann WP (2013) The relevance of the TGF-beta paradox to EMT-MET programs. Cancer Lett 341(1):30–40
16. Yao H, Baker D, Ten Dijke P (2019) TGF-β-mediated epithelial-mesenchymal transition and cancer metastasis. Int J Mol Sci 20(11):2767
17. Todaro GJ, Fryling C, De Larco JE (1980) Transforming growth factor factors produced by certain human tumor cells: polypeptides that interact with epidermal growth factor receptors. Proc Natl Acad Sci 77(9):5258–5262
18. Todaro GJ et al (1977) MSA and EGF receptors on sarcoma virus transformed cells and human fibrosarcoma cells in culture. Nature 267(5611):526–528
19. Zhao B, Chen Y-G (2014) Regulation of TGF-β signal transduction. Scientifica 2014:874065
20. Todaro GJ, De Larco JE, Cohen S (1976) Transformation by murine and feline sarcoma viruses specifically blocks binding of epidermal growth factor to cells. Nature 264(5581):26–31
21. de Larco JE, Todaro GJ (1978) Growth factors from murine sarcoma virus-transformed cells. Proc Natl Acad Sci 75(8):4001–4005
22. Roberts AB et al (1981) New class of transforming growth factors potentiated by epidermal growth factor: isolation from non-neoplastic tissues. Proc Natl Acad Sci 78(9):5339–5343
23. Moses HL, Roberts AB, Derynck R (2016) The discovery and early days of TGF-β: a historical perspective. Cold Spring Harb Perspect Biol 8(7):a021865
24. Fujii D et al (1986) Transforming growth factor beta gene maps to human chromosome 19 long arm and to mouse chromosome 7. Somat Cell Mol Genet 12(3):281–288
25. Barton DE et al (1988) Chromosomal mapping of genes for transforming growth factors beta and beta 3 in man and mouse. Identification of a major storage site, purification, and characterization. J Biol Chem 258(11):7155–7160
26. Heldin CH, Miyazono K, ten Dijke P (1997) TGF-β signalling from cell membrane to nucleus through SMAD proteins. Nature 390(6659):465–471
27. Miyazono K, Maeda S, Imamura T (2005) BMP receptor signaling: transcriptional targets, regulation of signals, and signaling cross-talk. Cytokine Growth Factor Rev 16(3):251–263
28. Hinck AP, Mueller TD, Springer TA (2016) Structural biology and evolution of the TGF-beta family. Cold Spring Harb Perspect Biol 8(12):e022103
29. Rifkin DB (2005) Latent transforming growth factor-beta (TGF-beta) binding proteins: orchestrators of TGF-beta availability. J Biol Chem 280(9):7409–7412
37. Annes JP et al (2004) Integrin alphaVbeta6-mediated activation of latent TGF-beta requires the latent TGF-beta binding protein-1. J Cell Biol 165(5):723–734
38. Chen Y et al (2002) Latent TGF-beta binding protein-3 (LTBP-3) requires binding to TGF-beta for secretion. FEBS Lett 517(1–3):277–280
39. Fjellbirkeland L et al (2003) Integrin alphavbeta8-mediated activation of transforming growth factor-beta inhibits human airway epithelial proliferation in intact bronchial tissue. Am J Pathol 163(2):533–542
40. Jenkins RG et al (2006) Ligation of protease-activated receptor 1 enhances alphav(betav) integrin-dependent TGF-beta activation and promotes acute lung injury. J Clin Invest 116(6):1606–1614
41. Shi M et al (2011) Latent TGF-beta structure and activation. Nature 474(7351):343–349
42. Crawford SE et al (1998) Thrombospondin-1 is a major activator of TGF-beta in vivo. Cell 93(7):1159–1170
43. Liénart S et al (2018) Structural basis of latent TGF-beta requires the latent TGF-beta binding protein-1. J Cell Biol 165(5):723–734
44. de Caestecker M (2004) The transforming growth factor-beta superfamily of receptors. Cytokine Growth Factor Rev 15(1):1–11
45. Kulkarni AB, Karlsson S (1993) Transforming growth factor-beta 1 knockout mice. A mutation in one cytokine gene causes a dramatic inflammatory disease. Am J Pathol 143(1):3
46. Dickson MC et al (1995) Defective haematopoiesis and vascularization in transforming growth factor beta 1 knockout mice. Development 121(6):1845–1854
47. Taya Y, O’Kane S, Ferguson M (1999) Pathogenesis of cleft palate in TGF-beta3 knockout mice. Development 126(17):3869–3879
48. Kaartinem V et al (1995) Abnormal lung development and cleft palate in mice lacking TGF–β1 indicates defects of epithelial–mesenchymal interaction. Nature Genet 11(4):415–421
49. Sanford LP et al (1997) TGFbeta2 knockout mice have multiple developmental defects that are non-overlapping with other TGF-beta knockout phenotypes. Development 124(13):2659–2670
50. Nawashad A et al (2007) TGFβ3 inhibits E-cadherin gene expression in palate medial-edge epithelial cells through a Smad2-Smad4-LEF1 transcription complex. J Cell Sci 120(9):1646–1653
51. Brown CB et al (1996) Antibodies to the type II TGFβ receptor block cell activation and migration during atrioventricular cushion formation in the heart. Dev Biol 174(2):248–257
52. Boyer AS, Runyan RB (2001) TGFbeta type III and TGFbeta type II receptors have distinctive activities during epithelial-mesenchymal cell transformation in the embryonic heart. Dev Dyn 221(4):454–459
53. Salib A (2017) Regulation of TGFβ2 expression during EMT in lung epithelial cells. Western Sydney University, Australia
54. Pelton RW et al (1991) Immunohistochemical localization of TGF beta 1, TGF beta 2, and TGF beta 3 in the mouse embryo: expression patterns suggest multiple roles during embryonic development. J Cell Biol 115(4):1091–1105
55. Akhurst RJ et al (1990) Transforming growth factor beta in mammalian embryogenesis. Prog Growth Factor Res 2(3):153–168
56. Memon MA et al (2008) Transforming growth factor beta (TGFβ1, TGFβ2 and TGFβ3) null-mutant phenotypes in embryonic gonadal development. Mol Cell Endocrinol 294(1–2):70–80
57. Bristow RE et al (1999) Altered expression of transforming growth factor-β ligands and receptors in primary and recurrent ovarian carcinoma. Cancer 85(3):658–668
58. Nilsson E et al (2001) Expression and action of transforming growth factor beta (TGFβ1, TGFβ2, TGFβ3) in normal bovine ovarian surface epithelium and implications for human ovarian cancer. Mol Cell Endocrinol 182(2):145–155
59. Zhao Y, Chegini N, Flanders KC (1994) Human fallopian tube expresses transforming growth factor (TGF beta) isoforms, TGF beta type I-III receptor messenger ribonucleic acid and protein, and contains [125I] TGF beta-binding sites. J Clin Endocrinol Metab 79(4):1177–1184
60. Dissen G et al (1994) Immature rat ovaries become revascularized rapidly after autotransplantation and show a gonadotropin-dependent increase in angiogenic factor gene expression. Endocrinology 134(3):1146–1154
61. Singh P et al (2018) Inhibin is a novel paracrine factor for tumor angiogenesis and metastasis. Cancer Res 78(11):2978–2989
62. Varadaraj A et al (2015) Epigenetic regulation of GDF2 suppresses anoikis in ovarian and breast epithelia. Neoplasia 17(11):826–838
63. Chang H-M, Leung PCK (2018) Physiological roles of activins in the human ovary. J Bio-X Res 1(3):111–119
64. Woodruff TK et al (1996) Inhibin A and inhibin B are inversely correlated to follicle-stimulating hormone, yet are discordant during the follicular phase of the rat estrous cycle, and inhibin A is expressed in a sexually dimorphic manner. Endocrinology 137(12):5463–5467
65. Hsueh AJ et al (1987) Heterodimers and homodimers of inhibin subunits have different paracrine action in the modulation of luteinizing hormone-stimulated androgen biosynthesis. Proc Natl Acad Sci U S A 84(14):5082–5086
66. Zhang Y et al (1996) Receptor-associated mad homologues synergize as effectors of the TGF-beta response. Nature 383(6596):168–172
67. Robertson IB, Rifkin DB (2016) Regulation of the bioavailability of TGF-β and TGF-β-related proteins. Cold Spring Harb Perspect Biol 8(6):a021907
68. Bai S, Cao X (2002) A nuclear antagonistic mechanism of inhibitory smads in transforming growth factor–beta signaling. J Biol Chem 277(6):4176–4182
69. Chang C (2016) Agonists and antagonists of TGF-β family ligands. Cold Spring Harb Perspect Biol 8(8):a021923
70. Gatza CE, Oh SY, Blobe GC (2010) Roles for the type III TGF-beta receptor in human cancer. Cell Signal 22(8):1163–1174
71. Cheifetz S et al (1987) The transforming growth factor-beta system, a complex pattern of cross-reactive ligands and receptors. Cell 48(3):409–415
72. Mittl PR et al (1996) The crystal structure of TGF-beta 3 and comparison to TGF-beta 2: implications for receptor binding. Protein Sci 5(7):1261–1271
73. Frick CL et al (2017) Sensing relative signal in the Tgf-beta/Smad pathway. Proc Natl Acad Sci U S A 114(14):E2975–E2982
74. Nayeem SM et al (2017) Residues of alpha helix H3 determine distinctive features of transforming growth factor β3. J Phys Chem B 121(22):5483–5498
75. Heldin C-H, Moustakas A (2016) Signaling receptors for TGF-β family members. Cold Spring Harb Perspect Biol 8(8):a022053
76. Holtzhausen A et al (2013) Novel bone morphogenetic protein 17(11):826–838
80. Goto K et al (2007) Selective inhibitory effects of Smad6 on bone morphogenetic protein type I receptors. J Biol Chem 282(28):20603–20611
81. Hanyu A et al (2001) The N domain of Smad7 is essential for specific inhibition of transforming growth factor-beta signaling. J Cell Biol 155(6):1017–1027
82. Massagué J (2012) TGFβ signalling in context. Nat Rev Mol Cell Biol 13(10):616–630
83. Ramachandran A et al (2018) TGF-beta uses a novel mode of receptor activation to phosphorylate SMAD1/5 and induce epithelial-to-mesenchymal transition. Elife 7:e31756
84. Liu IM et al (2009) TGFbeta-stimulated Smad1/5 phosphorylation requires the ALK5 L45 loop and mediates the pro-migratory TGFbeta switch. EMBO J 28(2):88–98
85. Shi Y et al (1998) Crystal structure of a Smad MH1 domain bound to DNA: insights on DNA binding in TGF-beta signaling. Cell 94(5):585–594
86. Wu RY et al (1997) Heteromeric and homomeric interactions correlate with signaling activity and functional cooperativity of Smad3 and Smad4/DPC4. Mol Cell Biol 17(5):2521–2528
87. Massague J (2012) TGFβ signaling in context. Nat Rev Mol Cell Biol 13(10):616–630
88. Alarcon C et al (2009) Nuclear CDKs drive Smad transcriptional activation and turnover in BMP and TGF-beta pathways. Cell 139(4):757–769
89. Gao S et al (2009) Ubiquitin ligase Nedd4L targets activated Smad2/3 to limit TGF-beta signaling. Mol Cell 36(3):457–468
90. Aragón E et al (2011) A Smad action turnover switch operated by WW domain readers of a phosphoserine code. Genes Dev 25(12):1275–1288
91. Liang J et al (2018) CDK8 selectively promotes the growth of colon cancer metastases in the liver by regulating gene expression of timp3 and matrix metalloproteinases. Cancer Res 78(23):6594–6606
92. Serrao A et al (2018) Mediator kinase CDK8/CDK19 drives YAP1-dependent BMP4-induced EMT in cancer. Oncogene 37(35):4792–4808
93. Vincent T et al (2009) A SNAIL1–SMAD3/4 transcriptional repressor complex promotes TGF-β-mediated epithelial–mesenchymal transition. Nature Cell Biol 11(8):943–950
94. Cao M et al (2012) MiR-23a regulates TGF-beta-induced epithelial-mesenchymal transition by targeting E-cadherin in lung cancer cells. Int J Oncol 41(3):869–875
95. Johansson J et al (2013) MiR-155-mediated loss of C/EBPβ shifts the TGF-beta response from growth inhibition to epithelial-mesenchymal transition, invasion and metastasis in breast cancer. Oncogene 32(50):5614–5624
96. Ma L et al (2010) miR-9, a MYC/MYCN-activated microRNA, regulates E-cadherin and cancer metastasis. Nature Cell Biol 12(3):247–256
97. Han X et al (2014) Critical role of miR-10b in transforming growth factor-β1-induced epithelial–mesenchymal transition in breast cancer. Cancer Gene Ther 21(2):60–67
98. Song Q et al (2014) miR-483–5p promotes invasion and metastasis of lung adenocarcinoma by targeting RhoGDH1 and ALCAM. Cancer Res 74(11):3031–3042
99. Sun C et al (2017) miR-34a mediates oxaliplatin resistance of colorectal cancer cells by inhibiting macroautophagy via transforming growth factor-β/Smad4 pathway. World J Gastroenterol 23(10):1816
100. Zhang X et al (2020) miR-324–5p inhibits gallbladder carcinoma cell metastatic behaviours by downregulation of transforming growth factor beta 2 expression. Artificial Cells Nanomed Biotechnol 48(1):315–324
101. Parikh A et al (2014) microRNA-181a has a critical role in ovarian cancer progression through the regulation of the epithelial–mesenchymal transition. Nature Commun 5:2977
102. Choi P-W, Ng S-W (2017) The functions of microRNA-200 family in ovarian cancer: beyond epithelial-mesenchymal transition. Int J Mol Sci 18(6):1207
103. Chaudhury A et al (2010) TGF-beta-mediated phosphorylation of hnRNP E1 induces EMT via transcript-selective translational induction of Dab2 and ILEI. Nature Cell Biol 12(3):286–293
104. Matouk IJ et al (2016) The role of the oncotelic H19 IncRNA in tumor metastasis: orchestrating the EMT-MET decision. Oncotarget 7(4):3748
105. Li J et al (2018) Long noncoding RNA H19 promotes transforming growth factor-β-induced epithelial–mesenchymal transition by acting as a competing endogenous RNA of miR-370-3p in ovarian cancer cells. Oncotargets Ther 11:427
106. Ma J, Xue M (2018) LINK-A IncRNA promotes migration and invasion of ovarian carcinoma cells by activating TGF-β pathway. Biosci Rep. https://doi.org/10.1042/BSR20180936
107. Mitra R et al (2017) Decoding critical long non-coding RNA in ovarian cancer epithelial-to-mesenchymal transition. Nat Commun 8(1):1604
108. Wang Y et al (2018) TGF-β-induced STAT3 overexpression promotes human head and neck squamous cell carcinoma invasion and metastasis through malat1/mir-30a interactions. Cancer Lett 436:52–62
109. Zhang J et al (2020) The long-noncoding RNA MALAT1 regulates TGF-β/Smad signaling through formation of a lncRNA-protein complex with Smads, SETD2 and PPM1A in hepatic cells. PLoS ONE 15(1):e0228160
110. Zhang X et al (2018) Long non-coding RNA PVT1 promotes epithelial-mesenchymal transition via the TGF-β/Smad pathway in pancreatic cancer cells. Oncology Rep 40(2):1093–1102
111. Shen Y et al (2017) A long non-coding RNA IncRNA-PE promotes invasion and epithelial–mesenchymal transition in hepato-cellular carcinoma through the mir-200a/b-ZEB1 pathway. Tumor Biology 39(5):1010428317705756
112. Gooding AJ et al (2019) The IncRNA BORG facilitates the genetic silencing of HIV-1 replication by displacing the polycomb repressive complex 2 from binding to the LTR promoter. Nucleic Acids Res 47(6):1207
113. Shen Y et al (2017) A long non-coding RNA IncRNA-PE promotes invasion and epithelial–mesenchymal transition in hepato-cellular carcinoma through the miR-200a/b-ZEB1 pathway. Tumor Biology 39(5):1010428317705756
114. Gooding AJ et al (2019) The IncRNA BORG: a novel inducer of TNBC metastasis, chemoresistance, and disease recurrence. J Cancer Metastasis Treat. https://doi.org/10.20517 /2394-4722.2019.11
115. Gooding AJ et al (2019) The IncRNA BORG facilitates the survival and chemoresistance of triple-negative breast cancers. Oncogene 38(12):2020–2041
116. Gooding AJ et al (2017) The IncRNA BORG drives breast cancer metastasis and disease recurrence. Sci Rep 7(1):1–18
117. Liang H et al (2018) LncRNA PTAR promotes EMT and invasion-metastasis in serous ovarian cancer by competitively binding miR-101–3p to regulate ZEB1 expression. Mol Cancer 17(1):1–13
118. Yue B et al (2016) LncRNA-ATB mediated E-cadherin repression promotes the progression of colon cancer and predicts poor prognosis. J Gastroenterol Hepatol 31(3):595–603
119. Qu D et al (2019) Long noncoding RNA MALAT1 promotes epithelial-mesenchymal transition via the TGF-beta/Smad pathway. J Exp Clin Cancer Res 38(1):107
120. Cheng J-T et al (2019) Insights into biological role of LncRNAs in epithelial-mesenchymal transition. Cells. https://doi.org/10.3390/cells8101178
121. Davidovich C, Cech TR (2015) The recruitment of chromatin modifiers by long noncoding RNAs: lessons from PRC2. RNA 21(12):2007–2022

122. Kumar S et al (2016) Activation of Mitofusin2 by Smad2-RNF1 complex during mitochondrial fusion. Mol Cell 62(4):520–531

123. Kim YS et al (2020) Context-dependent activation of SIRT3 is necessary for anchorage-independent survival and metastasis of ovarian cancer cells. Oncogene 39(8):1619–1633

124. Derynck R, Zhang YE (2003) Smad-dependent and Smad-independent pathways in TGF-beta family signalling. Nature 425(6958):577–584

125. Parvani JG, Taylor MA, Schiemann WP (2011) Noncanonical TGF-β signalings during mammary tumorigenesis. J Mammary Gland Biol Neoplasia 16(2):127–146

126. Oh SY et al (2013) The type III TGFbeta receptor regulates filopodia formation via a Cdc42-mediated IRSp53-N-WASP interaction in epithelial cells. Biochem J 454(1):79–89

127. Mythreye K, Blobe GC (2009) The type III TGF-beta receptor regulates epithelial and cancer cell migration through beta-arrestin2-mediated activation of Cdc42. Proc Natl Acad Sci U S A 106(20):8221–8226

128. Bakin AV et al (2002) p38 mitogen-activated protein kinase is required for TGF-beta-mediated fibroblast transdifferentiation and cell migration. J Cell Sci 115(15):3193–3206

129. Chapnick DA et al (2011) Partners in crime: the TGFβ and EMT. Mol Cell 42(17):3957–3967

130. Galliher AJ, Schiemann WP (2007) Src phosphorylates Tyr284 in Smad3 and induces Smad-independent, JNK-dependent apoptosis in ovarian cancer cells. Cell Signal 25(5):1245–1251

131. Lee MK et al (2007) TGF-beta activates Erk MAP kinase signalling through direct phosphorylation of ShcA. EMBO J 26(17):3957–3967

132. Galliher AJ, Schiemann WP (2007) Src phosphorylates Tyr284 in TGF-beta type II receptor and regulates TGF-beta stimulation of p38 MAPK during breast cancer cell proliferation and invasion. Cancer Res 67(8):3752–3758

133. Mulder KM (2000) Role of Ras and Mapks in TGFbeta signaling. Cytokine Growth Factor Rev 11(1–2):23–35

134. Xie L et al (2004) Activation of the Erk pathway is required for TGF-beta1-induced epithelial-mesenchymal transdifferentiation and a requirement for the ovary for abdominal dissemination. British J Cancer 122(8):1219–1230

135. Auer K et al (2015) Peritoneal tumor spread in serous ovarian cancer-epithelial mesenchymal status and outcome. Oncotarget 6(19):17261–17275

136. Risch HA et al (2001) Prevalence and penetrance of germline BRCA1 and BRCA2 mutations in a population series of 649 women with ovarian cancer. Am J Hum Genet 68(3):700–710

137. Li D et al (2015) BRCA1 regulates transforming growth factor-β (TGF-β1) signaling through Gadd45α by enhancing the protein stability of Smad4. Mol Oncol 9(8):1655–1666

138. Kindelberger DW et al (2007) Intraepithelial carcinoma of the fimbria and pelvic serous carcinoma: evidence for a causal relationship. Am J Surg Pathol 31(2):161–169

139. Przybycin CG et al (2010) Are all pelvic (nonuterine) serous carcinomas of tubal origin? Am J Surg Pathol 34(10):1407–1416

140. Verma A et al (2013) Transformation of the fallopian tube secretory epithelium leads to high-grade serous ovarian cancer in Brca;Tp53;Pten models. Cancer Cell 24(6):751–765

141. Labidi-Gal SY et al (2017) High grade serous ovarian carcinomas originate in the fallopian tube. Nature Commun 8(1):1093

142. Coffman LG et al (2016) New models of hematogenous ovarian cancer metastasis demonstrate preferential spread to the ovary and a requirement for the ovary for abdominal dissemination. Transl Res 175:92–102.e2

143. Irving-Rodgers HF, Harland ML, Rodgers RJ (2004) A novel basal lamina matrix of the stratified epithelium of the ovarian follicle. Matrix Biol 23(4):207–217

144. Korkut A et al (2018) A pan-cancer analysis reveals high-frequency genetic alterations in mediators of signaling by the TGF-beta superfamily. Cell Syst 7(4):422–437

145. Junhan Zhou WJ, Huang W, Ye M, Zhu X (2020) Prognostic values of transforming growth factor-betasubtypes in ovarian cancer. BioMed Res Int. https://doi.org/10.1155/2020/2170606

146. Abendstein B et al (2000) Regulation of transforming growth factor-β secretion by human peritoneal mesothelial and ovarian carcinoma cells. Cytokine 12(7):1115–1119

147. Momenimovahed Z et al (2019) Ovarian cancer in the world: epidemiology and risk factors. Int J Womens Health 11:287–299

148. Kurman RJ, Shih Ie M (2010) The origin and pathogenesis of epithelial ovarian cancer: a proposed unifying theory. Am J Surg Pathol 34(3):433–443

149. Masoodi T et al (2020) Genetic heterogeneity and evolutionary history of high-grade ovarian carcinoma and matched distant metastases. British J Cancer 122(8):1219–1230

150. Coscia F et al (2016) Integrative proteomic profiling of ovarian cancer cell lines reveals precursor cell associated proteins and functional status. Nature Commun 7(1):1–14

151. Ahmed AA et al (2010) Driver mutations in TP53 are ubiquitous in high grade serous carcinoma of the ovary. J Pathol 221(1):49–56

152. Masoodi T et al (2020) Genetic heterogeneity and evolutionary history of high-grade ovarian carcinoma and matched distant metastases. British J Cancer 122(8):1219–1230

153. Konecny GE et al (2014) Prognostic and therapeutic relevance of molecular subtypes in high-grade serous ovarian cancer. JNCI. https://doi.org/10.1093/jnci/dju249

154. Kindelberger DW et al (2007) Intraepithelial carcinoma of the fimbria and pelvic serous carcinoma: evidence for a causal relationship. Am J Surg Pathol 31(2):161–169

155. Konecny GE et al (2014) Prognostic and therapeutic relevance of molecular subtypes in high-grade serous ovarian cancer. JNCI. https://doi.org/10.1093/jnci/dju249

156. Kindelberger DW et al (2007) Intraepithelial carcinoma of the fimbria and pelvic serous carcinoma: evidence for a causal relationship. Am J Surg Pathol 31(2):161–169

157. Konecny GE et al (2014) Prognostic and therapeutic relevance of molecular subtypes in high-grade serous ovarian cancer. JNCI. https://doi.org/10.1093/jnci/dju249
167. Blechsmit K et al (2008) The E-cadherin repressor Snail is associated with lower overall survival of ovarian cancer patients. British J Cancer 98(2):489–495
168. Shim HS, Yoon BS, Cho NH (2009) Prognostic significance of paired epithelial cell adhesion molecule and E-cadherin in ovarian serous carcinoma. Human Pathol 40(5):693–698
169. Knarr M et al (2020) miR-181a initiates and perpetuates oncogenic transformation through the regulation of innate immune signaling. Nature Commun 11(1):3231
170. Bakhoun SF et al (2018) Chromosomal instability drives metastasis through a cytotoxic DNA response. Nature 553(7689):467–472
171. Papageorgis P, Stylianopoulos T (2015) Role of TGFβ in regulation of the tumor microenvironment and drug delivery. Int J Oncol 46(3):933–943
172. Metelli A et al (2016) Surface expression of TGFβ docking receptor GARP promotes oncogenesis and immune tolerance in breast cancer. Cancer Res 76(24):7106–7117
173. Wang H et al (2013) Epithelial–mesenchymal transition (EMT) induced by TNF-α requires AKT/GSK-3β-mediated stabilization of snail in colorectal cancer. PLoS ONE 8(2):e56664
174. Joseph JV et al (2015) Hypoxia enhances migration and invasion in glioblastoma by promoting a mesenchymal shift mediated by the HIF1α–ZEB1 axis. Cancer Lett 359(1):107–116
175. Overstreet JM et al (2014) Redox control of p53 in the transcriptional regulation of TGF-β1 target genes through SMAD cooperativity. Cell Signal 26(7):1427–1436
176. Jaffer OA et al (2015) Mitochondrial-targeted antioxidant therapy decreases transforming growth factor-β–mediated collagen production in a murine asthma model. Am J Respir Cell Mol Biol 52(1):106–115
177. Pociask DA, Sime PJ, Brody AR (2004) Asbestos-derived reactive oxygen species activate TGF-β 1. Lab Invest 84(8):1013–1023
178. Mikula-Pietrasik J et al (2018) The peritoneal “seed” for a cancerous “soil”: a comprehensive review of the pathogenesis of intra-peritoneal cancer metastases. Cell Mol Life Sci 75(3):509–525
179. Wang E et al (2005) Peritoneal and subperitoneal stroma may facilitate regional spread of ovarian cancer. Clin Cancer Res 11(1):113
180. Burleson KM et al (2004) Ovarian carcinoma ascites spheroids adhere to extracellular matrix components and mesothelial cell monolayers. Gynecol Oncol 93(1):170–181
181. Kenny HA et al (2014) Mesothelial cells promote early ovarian cancer metastasis through fibronectin secretion. J Clin Invest 124(10):4614–4628
182. He P, Qiu K, Jia Y (2018) Modeling of mesenchymal hybrid epithelial state and phenotypic transitions in EMT and MET processes of cancer cells. Sci Rep 8(1):14323
183. López-Cabrera M (2014) Mesenchymal conversion of mesothelial cells is a key event in the pathophysiology of the peritoneum during peritoneal dialysis. Adv Med 2014:473134
184. Strippoli P et al (2020) Cavelin1 and YAP drive mechanically induced mesothelial to mesenchymal transition and fibrosis. Cell Death Dis 11(8):647
185. Young VJ et al (2014) The peritoneum is both a source and target of TGF-β in women with endometriosis. PLoS ONE 9(9):e106773
186. Sandoval P et al (2013) Carcinoma-associated fibroblasts derive from mesothelial cells via mesothelial-to-mesenchymal transition in peritoneal metastasis. J Pathol 231(4):517–531
187. Gao Q et al (2019) Heterotypic CAF-tumor spheroids promote early peritoneal metastasis of ovarian cancer. J Exp Med 216(3):688–703
188. Heath RM et al (2004) Tumour-induced apoptosis in human mesothelial cells: a mechanism of peritoneal invasion by Fas Ligand/Fas interaction. British J Cancer 90(7):1437–1442
189. Iwaniicki MP et al (2011) Ovarian cancer spheroids use myosin-generated force to clear the mesothelium. Cancer Discov 1(2):144
190. Liu Y et al (2017) YAP modulates TGF-β1-induced simultaneous apoptosis and EMT through upregulation of the EGF receptor. Sci Rep 7(1):45523
191. Yang Y et al (2006) Transforming growth factor-beta induces epithelial-to-mesenchymal transition and apoptosis via a cell cycle-dependent mechanism. Oncogene 25(55):7235–7244
192. Lane D et al (2015) Inflammation-regulating factors in ascites as predictive biomarkers of drug resistance and progression-free survival in serous epithelial ovarian cancers. BMC Cancer 15(1):492
193. Feigenberg T et al (2014) Molecular profiling and clinical outcome of high-grade serous ovarian cancer presenting with low versus high-volume ascites. Biomed Res Int 2014:367103
194. Ford CE et al (2020) The untapped potential of ascites in ovarian cancer research and treatment. British J Cancer 123(1):9–16
195. Lee CK et al (2019) Development and validation of a prognostic nomogram for overall survival in patients with platinum-resistant ovarian cancer treated with chemotherapy. Eur J Cancer 117:99–106
196. Adam RA, Adam YG (2004) Malignant ascites: past, present, and future. J Am Coll Surg 198(6):999–1011
197. Nagy JA et al (1993) Pathogenesis of malignant ascites formation: initiating events that lead to fluid accumulation. Cancer Res 53(11):2631–2643
198. Liao S et al (2011) TGF-beta blockade controls ascites by preventing abnormalization of lymphatic vessels in orthotopic human ovarian carcinoma models. Clin Cancer Res 17(6):1415–1424
199. Santin AD et al (2001) Increased levels of interleukin-10 and transforming growth factor-beta in the plasma and ascitic fluid of patients with advanced ovarian cancer. BJOG 108(8):804–808
200. Yang L et al (2017) Ascites promotes cell migration through the repression of miR-125b in ovarian cancer. Oncotarget 8(31):51008–51015
201. Sheid B (1992) Angiogenic effects of macrophages isolated from ascitic fluid aspirated from women with advanced ovarian cancer. Cancer Lett 62(2):153–158
202. Kan T et al (2020) Single-cell EMT-related transcriptional analysis revealed intra-cluster heterogeneity of tumor cell clusters in epithelial ovarian cancer ascites. Oncogene 39(21):4227–4240
203. Pakula M et al (2019) A unique pattern of mesothelial-mesenchymal transition induced in the normal peritoneal mesothelium by high-grade serous ovarian cancer. Cancers 11(5):662
204. Paoli P, Giannoni E (1833) Anoikis molecular pathways and its role in cancer progression. Biochimica et Biophysica Acta (BBA) Mol Cell Res 12:3481–3498
205. Li X, Wang X (2017) The emerging roles and therapeutic potential of exosomes in epithelial ovarian cancer. Mol Cancer 16(1):92
206. Webber J et al (2010) Cancer exosomes trigger fibroblast to myofibroblast differentiation. Cancer Res 70(23):9621–9630
207. Mashouri L et al (2019) Exosomes: composition, biogenesis, and mechanisms in cancer metastasis and drug resistance. Mol Cancer 18(1):75
208. Cornel L et al (2019) MicroRNA-mediated suppression of the TGF-β pathway confers transmissible and reversible CDK4/6 inhibitor resistance. Cell Rep 26(10):2667–80.e7
209. Graves LE et al (2004) Proinvasive properties of ovarian cancer ascites-derived membrane vesicles. Cancer Res 64(19):7045–7049
Clinical & Experimental Metastasis (2021) 38:139–161

210. Neill T et al (2013) Decorin induces rapid secretion of thrombospondin-1 in basal breast carcinoma cells via inhibition of Ras homolog gene family, member A/Rho-associated coiled-coil containing protein kinase 1. FEBS J 280(10):2353–2368

211. Yung S et al (1995) Source of peritoneal proteoglycans. Human peritoneal mesothelial cells synthesize and secrete mainly small dermatan sulfate proteoglycans. Am J Pathol 146(2):520–529

212. Nieman KM et al (2013) Adipocytes promote ovarian cancer metastasis and provide energy for rapid tumor growth. Nature Med 17(11):1498

213. Etzerodt A et al (2020) Tissue-resident macrophages in omentum promote metastatic spread of ovarian cancer. J Exp Med 217(4):e20191869

214. McNally L et al (2015) Does omentectomy in epithelial ovarian cancer affect survival? An analysis of the surveillance, epidemiology, and results database. Int J Gynecol Cancer 25(4):607–615

215. Motohara T et al (2019) An evolving story of the metastatic voyage of ovarian cancer cells: cellular and molecular orchestration of the adipose-rich metastatic microenvironment. Oncogene 38(16):2885–2898

216. Shiomotsuma M et al (1993) Morpho-physiological function and role of omental milky spots as omentum-associated lymphoid tissue (OALT) in the peritoneal cavity. Lymphology 26(2):90–101

217. Cai J et al (2012) Fibroblasts in omentum activated by tumor cells promote ovarian cancer growth, adhesion and invasiveness. Carcinogenesis 33(1):20–29

218. Cheon DJ et al (2014) A collagen-remodeling gene signature regulated by TGF-β signaling is associated with metastasis and poor survival in serous ovarian cancer. Clin Cancer Res 20(3):711–723

219. Huang YL et al (2020) Collagen-rich omentum is a premetastatic niche for integrin α2-mediated peritoneal metastasis. Elife 9:e59442

220. Meza-Perez S, Randall TD (2017) Immunological functions of the omentum. Trends Immunol 38(7):526–536

221. Gratchev A (2017) TGF-βa signalling in tumour associated macrophages. Immunobiology 222(1):75–81

222. Ho M-Y et al (2012) TNF-α induces epithelial–mesenchymal transition of renal cell carcinoma cells via a GSK3β-dependent mechanism. Mol Cancer Res 10(8):1109–1119

223. Fu X-T et al (2015) Macrophage-secreted IL-8 induces epithelial-mesenchymal transition in hepato/pancreatic carcinoma cells by activating the JAK2/STAT3/Snail pathway. Int J Oncol 46(2):587–596

224. Nieman KM et al (1831) Adipose tissue and adipocytes support tumorigenesis and metastasis. Biochimica et Biophysica Acta (BBA) Mol Cell Biol Lipids 10:1533–1541

225. Tucker SL et al (2014) Molecular biomarkers of residual disease after surgical debulking of high-grade serous ovarian cancer. Clin Cancer Res 20(12):3280–3288

226. Jin J et al (2018) Fatty acid binding protein 4 promotes epithelial-mesenchymal transition in cervical squamous cell carcinoma through AKT/GSK3β/Snail signaling pathway. Mol Cell Endocrinol 461:155–164

227. Dirat B et al (2011) Cancer-associated adipocytes exhibit an activated phenotype and contribute to breast cancer invasion. Cancer Res 71(7):2455–2465

228. Park J, Morley TS, Scherer PE (2013) Inhibition of endotrophin, a cleavage product of collagen VI, confers cisplatin sensitivity to tumours. EMBO Mol Med 5(6):935–948

229. Ishay-Ronen D, Christofori G (2019) Targeting cancer cell metastasis by converting cancer cells into fat. Cancer Res 79(21):5471

230. Ledermann J et al (2014) Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. Lancet Oncol 15(8):852–861

231. Bookman M, et al. (2017) Harmonising clinical trials within the gynecologic cancer intergroup: consensus and unmet needs from the Fifth Ovarian Cancer Consensus Conference. Ann Oncol 28(suppl_8):viii30–viii5

232. Deng J et al (2016) Targeting epithelial-mesenchymal transition and cancer stem cells for chemoresistant ovarian cancer. Onco-target 7(34):55771

233. Steg AD et al (2012) Stem cell pathways contribute to clinical chemoresistance in ovarian cancer. Clin Cancer Res 18(3):869–881

234. Smith AG, Macleod KF (2019) Autophagy, cancer stem cells and drug resistance. J Pathol 247(5):708–718

235. Saxena M et al (2011) Transcription factors that mediate epithelial-mesenchymal transition lead to multidrug resistance by upregulating ABC transporters. Cell Death Dis 2(7):e179

236. Srivastava AK et al (2015) Enhanced expression of DNA polymerase eta correlates to cisplatin resistance of ovarian cancer stem cells. Proc Natl Acad Sci USA 112(14):4411–4416

237. Mitra T et al (2018) Stemness and chemoresistance are imparted to the OC cells through TGFβ1 driven EMT. J Cell Biochem 119(7):5775–5787

238. Huang S et al (2012) MED12 controls the response to multiple cancer drugs through regulation of TGF-β receptor signaling. Cell 151(5):937–950

239. Davis FM et al (2014) Targeting EMT in cancer: opportunities for pharmaceutical intervention. Trends Pharmacol Sci 35(9):479–488

240. Yang D et al (2013) Integrated analyses identify a master microRNA regulatory network for the mesenchymal subtype in serous ovarian cancer. Cancer Cell 23(2):186–199

241. Li X et al (2016) Preclinical efficacy and safety assessment of artemisinin-chemotherapeutic agent conjugates for ovarian cancer. EBioMedicine 14:44–54

242. Buïjs JT et al (2007) Bone morphogenetic protein 7 in the development and treatment of bone metastases from breast cancer. Cancer Res 67(18):8742–8751

243. Buïjs JT et al (2007) BMP7, a putative regulator of epithelial homeostasis in the human prostate, is a potent inhibitor of prostate cancer bone metastasis in vivo. Am J Pathol 171(3):1047–1057

244. Ishay-Ronen D et al (2019) Gain fat—lose metastasis: converting invasive breast cancer cells into adipocytes inhibits cancer metastasis. Cancer Cell 35(1):17–32

245. Mathow D et al (2015) Zeb1 affects epithelial cell adhesion by diverting glycosphingolipid metabolism. EMBO Rep 16(3):321–331
251. Schwab A et al (2018) Polyl pathway links glucose metabolism to the aggressiveness of cancer cells. Cancer Res 78(7):1604–1618
252. Brown JR et al (2020) Phase II clinical trial of metformin as a cancer stem cell–targeting agent in ovarian cancer. JCI Insight 5(11):e143247
253. Anderton MJ et al (2011) Induction of heart valve lesions by small-molecule ALK5 inhibitors. Toxicol Pathol 39(6):916–924
254. Battle E, Massagué J (2019) Transforming growth factor-β signaling in immunity and cancer. Immunity 50(4):924–940
255. Morris JC et al (2014) Phase I study of GC1008 (fresolimumab): a human anti-transforming growth factor-beta (TGFβ) monoclonal antibody in patients with advanced malignant melanoma or renal cell carcinoma. PLoS ONE 9(3):e90353
256. Formenti SC et al (2018) Focal irradiation and systemic TGFβ blockade in metastatic breast cancer. Clin Cancer Res 24(11):2493–2504
257. Tauer JT, Abdullah S, Rauch F (2019) Effect of anti-TGF-beta treatment in a mouse model of severe osteogenesis imperfecta. J Bone Miner Res 34(2):207–214
258. Lee SW et al (2016) Bone mineral density in women treated for various types of gynecological cancer. Asia-Pacific J Clin Oncol 12(4):e398–e404
259. D’Cruz O et al (2017) Transforming growth factor-beta 2 (TGF-β2) antisense oligonucleotide (ASO) OT-101 synergizes with chemotherapy in preclinical tumor models. AACC: https://doi.org/10.1158/1538-7445.AM2017-2800
260. Van Aarsen LA et al (2008) Antibody-mediated blockade of integrin alpha v beta 6 inhibits tumor progression in vivo by a transforming growth factor-beta-regulated mechanism. Cancer Res 68(2):561–570
261. de Steer G et al (2020) Selective inhibition of TGF-beta1 produced by GARP-expressing tregs overcomes resistance to PD-L1/PD-L1 blockade in cancer. Nat Commun 11(1):4545
262. Kim SK et al (2017) An engineered transforming growth factor β (TGF-β) monomer that functions as a dominant negative to block TGF-β signaling. J Biol Chem 292(17):7173–7188
263. Gallo-Oller G et al (2016) PI44, a transforming growth factor beta inhibitor peptide, generates antitumoral effects and modifies SMAD7 and SKI levels in human glioblastoma cell lines. Cancer Lett 381(1):67–75
264. Mendoza V et al (2009) Betaglycan has two independent domains required for high affinity TGF-β binding: proteolytic cleavage separates the domains and inactivates the neutralizing activity of the soluble receptor. Biochemistry 48(49):11755–11765
265. Tao JJ et al (2019) First-in-human phase I study of the activin A inhibitor, STM 434, in patients with granulosa cell ovarian cancer and other advanced solid tumors. Clin Cancer Res 25(18):5458
266. Sheen YY et al (2013) Targeting the transforming growth factor-β signaling in cancer therapy. Biomol Ther 21(5):323–331
267. Zhang Q et al (2018) NY-LI257299 monohydrate, a TGF-βRI inhibitor, suppresses tumor growth and ascites development in ovarian cancer. Cancers 10(8):260
268. Connolly EC et al (2011) Outgrowth of drug-resistant carcinomas expressing markers of tumor aggressiveness after long-term TβRI/II kinase inhibition with LY2109761. Cancer Res 71(6):2339–2349
269. Spender LC et al (2019) Preclinical evaluation of AZ12601011 and AZ12799734, inhibitors of transforming growth factor β superfamily type 1 receptors. Mol Pharmacol 95(2):222–234
270. Newsted D et al (2019) Blockade of TGF-β signaling with novel synthetic antibodies limits immune exclusion and improves chemotherapy response in metastatic ovarian cancer models. Oncoimmunology 8(2):e1539613
271. Yao E-H et al (2009) A pyrrole–imidazole polyamide targeting transforming growth factor-β1 inhibits restenosis and preserves endothelialization in the injured artery. Cardiovasc Res 81(4):797–804
272. Chen M et al (2010) Pretranscriptional regulation of Tgf-β1 by PI polyamide prevents scarring and accelerates wound healing of the cornea after exposure to alkali. Mol Ther 18(3):519–527
273. Matsuda H et al (2011) Transcriptional inhibition of progressive renal disease by gene silencing pyrrole–imidazole polyamide targeting of the transforming growth factor-β1 promoter. Kidney Int 79(1):46–56
274. Washio H et al (2011) Transcriptional inhibition of hypertrophic scars by a gene silencer, pyrrole–imidazole polyamide, targeting the TGF-β1 promoter. J Invest Dermatol 131(10):1987–1995
275. Igarashi J et al (2015) Preclinical study of novel gene silencer pyrrole-imidazole polyamide targeting human TGF-β1 promoter for hypertrophic scars in a common marmoset primate model. PLoS ONE 10(5):e0125295
276. Sow HS et al (2019) Combined inhibition of TGF-β signaling and the PD-L1 immune checkpoint is differentially effective in tumor models. Cells 8(4):320
277. Mariathasan S et al (2018) TGFβ attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. Nature 554(7693):544–548
278. Lan Y et al (2018) Enhanced preclinical antitumor activity of M7824, a bifunctional fusion protein simultaneously targeting PD-L1 and TGF-beta. Sci Transl Med. https://doi.org/10.1126/scitranslmed.aan5448
279. Wang L et al (2010) Immunotherapy for human renal cell carcinoma by adoptive transfer of autologous transforming growth factor β–insensitive CD8+ T cells. Clin Cancer Res 16(1):164–173
280. Fakhrai H et al (2006) Phase I clinical trial of a TGF-β antisense-modified tumour cell vaccine in patients with advanced glioma. Cancer Gene Ther 13(12):1052–1060
281. Olivares J et al (2011) Phase I trial of TGF-β2 antisense GM-CSF gene-modified autologous tumor cell (TAG) vaccine. Clin Cancer Res 17(1):183–192
282. Faivre S et al (2019) Novel transforming growth factor beta receptor I kinase inhibitor galunisertib (LY2157299) in advanced hepatocellular carcinoma. Liver Int 39(8):1468–1477
283. Morris JC et al (2014) Phase I study of GC1008 (fresolimumab): a human anti-transforming growth factor-beta(TGFBeta) monoclonal antibody in patients with advanced malignant melanoma or renal cell carcinoma. PLoS ONE 9(3):e90353
284. Cohn A et al (2014) A phase I dose-escalation study to a predefined dose of a transforming growth factor-beta1 monoclonal antibody (BetaM1) in patients with metastatic cancer. Int J Oncol 45(6):2221–2231
285. Trials.gov c Phase I/IIb study of NIS793 in combination With PDRO01 in patients with advanced malignancies. ClinicalTrialsgov Identifier: NCT02947165
286. Bogdahn U et al (2011) Targeted therapy for high-grade glioma with the TGF-beta2 inhibitor trabedersen: results of a randomized and controlled phase IIb study. Neuro Oncol 13(1):132–142
287. Trials.gov C Study to determine the safety, tolerability, pharmacokinetics and RP2D of ABBV-151 as a single agent and in combination with ABBV-181 in participants with locally advanced or metastatic solid tumors. ClinicalTrialsgov Identifier: NCT03821935
288. Timothy Yap1 DA, Debra Wood3, Jean-François Denis3, Tina Gruosso3, Gilles Tremblay3, Maureen O’Connor-McCourt3, Nehal Lakhani4 P856 AVID200, first-in-class TGF-beta1 and small-molecule ALK5 inhibitors. Toxicol Pathol 39(6):916–924
289. Vicki Leigh Keedy TMB, Jeffrey Melson Clarke, Herbert Hurwitz, Insun Baek, Ilho Ha, Chan-Young Ock, Su Youn Nam,
Mina Kim, Neunggyu Park, Jung Yong Kim, Seong-Jin Kim
Association of TGF-β responsive signature with anti-tumor effect
of vactosertib, a potent, oral TGF-β receptor type I (TGFBRI)
inhibitor in patients with advanced solid tumor. J Clin Oncol 36,
no 15_suppl

290. Goff LW et al (2016) A phase I study of the anti-activin recep-
tor-like kinase 1 (ALK-1) monoclonal antibody PF-03446962
in patients with advanced solid tumors. Clin Cancer Res
22(9):2146–2154

291. Nemunaitis J et al (2006) Phase II study of belagenpumatumucel-
L, a transforming growth factor beta-2 antisense gene-modified
allogeneic tumor cell vaccine in non-small-cell lung cancer. J
Clin Oncol 24(29):4721–4730

292. Olivares J et al (2011) Phase I trial of TGF-beta 2 antisense GM-
CSF gene-modified autologous tumor cell (TAG) vaccine. Clin
Cancer Res 17(1):183–192

Publisher’s Note Springer Nature remains neutral with regard to
jurisdictional claims in published maps and institutional affiliations.