

REVIEW

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Targeting tumour microenvironment by tyrosine kinase inhibitor

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Abstract

Tumour microenvironment (TME) is a key determinant of tumour growth and metastasis. TME could be very different for each type and location of tumour and TME may change constantly during tumour growth. Multiple counterparts in surrounding microenvironment including mesenchymal-, hematopoietic-originated cells as well as non-cellular components affect TME. Thus, therapeutics that can disrupt the tumour-favouring microenvironment should be further explored for cancer therapy. Previous efforts in unravelling the dysregulated mechanisms of TME components has identified numerous protein tyrosine kinases, while its corresponding inhibitors have demonstrated potent modulatory effect on TME. Recent works have demonstrated that beyond the direct action on cancer cells, tyrosine kinase inhibitors (TKIs) have been implicated in inactivation or normalization of dysregulated TME components leading to cancer regression. Either through re-sensitizing the tumour cells or reversing the immunological tolerance microenvironment, the emergence of these TME modulatory mechanism of TKIs supports the combinatory use of TKIs with current chemotherapy or immunotherapy for cancer therapy. Therefore, an appropriate understanding on TME modulation by TKIs may offer another mode of action of TKIs for cancer treatment. This review highlights mode of kinase activation or paracrine ligand production from TME components and summarises the findings on the potential use of various TKIs on regulating TME components. At last, the combination use of current TKIs with immunotherapy in the perspectives of efficacy and safety are discussed.

Keywords: Tumour microenvironment, Tyrosine kinase inhibitors, Immunotherapy

Background

Protein phosphorylation, one of the most prevalent post-translational modification of protein, is tightly regulated by specific protein kinase that transfer phosphate group to the amino acid residue [1]. To our knowledge, there are in total 518 kinases in human genome [2], while 90 of them are classified in the category of tyrosine kinases (58 of them are receptor tyrosine kinase RTK; the remaining are non-receptor tyrosine kinase) [3]. Specifically, tyrosine kinase transfers phosphate group from ATP to tyrosine residue of the protein. In carcinogenesis, the aberrant activation of protein phosphorylation, particularly by RTKs has been frequently described [4]. Mutation or gene amplification of tyrosine kinase signalling further promote the carcinogenesis process,

including survival, proliferation, motility, and metabolism as well as escape from immune surveillance [5]. For instances, overexpression of epidermal growth factor receptor (EGFR) and platelet derived growth factor receptor α/β (PDGFR) have been well implicated in supporting various malignancy growth and progression [6, 7].

The binding of ligand such as growth factors or cytokines to the extracellular domains of RTKs initiate the signalling cascade by changing its structure and kinase activation. Whereas the non-RTKs which lack of extracellular domains mainly serves as the downstream effector of RTKs [8]. Understanding on the mode of action of tyrosine kinases had led to discovery of many small molecule inhibitors proved to be effective for the cancer treatment. To date, more than 30 RTK inhibitors have been approved by US FDA. They can inhibit single target or multiple targets. For instances, gefitinib and erlotinib which primarily inhibit EGFR, is used for EGFR-

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mutated lung cancer patients [9]. Imatinib mesylate has multiple targets, c-KIT, PDGFR and c-ABL, is indicated for acute and chronic myeloid leukemia as well as gastrointestinal stromal patients [10, 11]; sorafenib which multi-targeted VEGFR, PDGFR and Raf, is utilized for advanced renal cell carcinoma and hepatocellular carcinoma patients [12, 13]. Much previous efforts on tyrosine kinase inhibitors (TKIs) have focused on their direct actions in regulating tumour growth and angiogenesis, while recent emerging studies have re-focused on how TKIs modulate the tumour microenvironment (TME). An appropriate understanding on how TKIs alter the TME may help pave another mode of action of TKIs in cancer therapy. To maximally exploit the therapeutic benefit of current tyrosine kinase inhibitors, we systematically searched through the PubMed database with the MeSH terms of “tumour microenvironment” and “Protein-Tyrosine Kinases/antagonists and inhibitors”. In this review, we highlight the impact of kinase activation and paracrine production of ligand from tumour stroma, and summarise the findings on the potential effect of various type of tyrosine kinase inhibitors on tumour microenvironment components which including mesenchymal cells, hematopoietic cells and non-cellular components. The perspective of combination use of current kinase inhibitor with immunotherapy and modulation of TME in overcoming TKIs resistance for cancer treatment are also discussed.

Targeting tumour microenvironment with TKI

Kinase inhibition in tumour cells by tyrosine kinase inhibitors offers promising clinical benefit to cancer patients, especially, who have tumour with mutated kinases [14]. Nonetheless, aberrant activation of tumour microenvironment may fail therapeutics that are merely targeting on cancer cells. The fact that tumour cells can promote their growth by recruiting and communicating with other type of cells, such as mesenchymal- and hematopoietic-originated cells, in the tumour microenvironment (TME) [15]. Given the accumulating evidences of tyrosine kinase paracrine receptor activation or ligand production by TME components, TKIs may be a promising TME modulator apart from regulating the intrinsic functions of tumour cell [16–18]. Taken the example of tumour immunology, TKIs generally act on the two mechanisms: immunogenic control and immune conditioning [19]. Immunogenic control is the modulation of tumour cells sensitivity in response to alteration of immunosuppressive phenotype, whereas immune conditioning is the direct changes of TKIs incurred on the function and number of immune cells populations. As detailed below, TKIs have been implicated in essentially all components of TME and thus increasingly recognised as a promising candidate for TME modulation.

Targeting the mesenchymal cells

Endothelial cells

Endothelial cells are known to play pivotal role in tumour neovascularization. The role of TKIs in endothelial cells has been identified either through direct action on endothelial cells or through mediating the communication between endothelial cells and tumour cells. Earlier studies observed the PKI 166, epidermal growth factor receptor (EGFR) inhibitor attenuated EGFR activation in tumour associated endothelial cells and caused cell apoptosis. The action is deemed to be EGFR-specific as it was not observed on EGFR-negative mice [20]. Subsequent findings on erlotinib and gefitinib, EGFR inhibitors showed combination use of TKI with chemotherapeutic agents enhanced vessel permeability and fractional plasma volume, which consequently, optimized the efficacy of drug delivery [21, 22]. Endothelial cells secreted-EGF promoted the migration property of T-cadherin deficient cancer cells, which was found to be more sensitive to EGF response. Gefitinib treatment blocked the cancer cell migration, suggestive of the negative correlation of cancer cells-expressing T-cadherin and EGFR activation on endothelial cells [23]. More specifically, gefitinib reduced the coverage of endothelial cells and perivascular cells as well the recruitment of perivascular progenitor cells into tumour region [24]. Moreover, endothelial cells are known to express receptor tyrosine kinases vascular endothelial growth factors (VEGFR) and platelet-derived growth factors (PDGFR), in which binding of VEGF or PDGF to VEGFR and PDGFR on endothelial cells elicits endothelial cells proliferation, migration and tumour neovascularization [25]. Thus, there has been considerable efforts in targeting VEGFR and PDGFR on endothelial cells as ideal therapeutic target for tumour neovascularization. For instances, SU5416, the VEGFR2 inhibitor eliminated melanoma microvasculature [26]; imatinib mesylate, the PDGFR inhibitor blocked the angiogenesis in prostate cancer bone metastases [27]; Ki8751, the VEGFR inhibitor reversed MDR1 up-regulation and endothelial cells chemoresistance [28].

Given that several target receptors act as endothelial cell regulators, multi-targeted tyrosine kinase inhibitors are also being evaluated for better therapeutic outcome. The dual tyrosine kinase inhibitor AEE788 is utilized to inhibit EGFR and VEGFR binding on endothelial cells and resulted in apoptosis in both tumour and endothelial cells [29]. The intervention of pazopanib that targeted on PDGFR, VEGFR and KIT receptors in endothelial cells enhanced chemo-sensitivity of cancer cells to chemotherapeutic treatment [30]. Sunitinib, the inhibitor for VEGFR and PDGFR inhibited tube formation and outgrowth of aortic ring [31] as well as induced endothelial cells death [32]. Observing the shared constitutively activated tyrosine kinases of

PDGFR and Src, the use of dasatinib, the Src/Abl kinase inhibitor also deferred tumour angiogenesis [33]. Taken together, aiming at PDGFR and VEGFR on endothelial cells and pericytes appear to be an ideal target for vessel normalization; however, imatinib and sunitinib treatments have also shown to increase the dissemination of tumour cells. Recent finding demonstrated that vasculature-supporting pericytes exhaustion enhanced breast-to-lung metastasis, which is associated with shift of hypoxia and epithelial-to-mesenchymal phenotype. Therefore, suppressing pericyte coverage with imatinib and sunitinib further increased the metastatic rate followed by enhanced hypoxia and mesenchymal phenotype in tumour-bearing mice [34]. By targeting on endothelial cells and pericytes which preserve the vessel integrity, the combination use of TKIs with other interventions could be a better approach to achieve the optimal tumour inhibitory function. For instance, the MET inhibitor PF2341066 was administrated with imatinib or sunitinib to reduce the epithelial-to-mesenchymal transition [15] and co-administration of imatinib with osteoclast-depleting zoledronate declined prevalence of lymph node metastasis [35].

Fibroblast

Cancer associated stromal cell, comprised of approximately 40% of total tumour volume [36], plays a pivotal role in carcinogenesis, in which its role involves promoting cancer cell initiation [37], extracellular matrix remodelling [38], and even preserving stem/progenitor cells [39]. For instances, mesenchymal stromal cells protect the stem/progenitor cells from TKI-induced apoptosis, thereby leading to reduced sensitivity of stem cells towards TKI exposure and tumour relapse [39]. The negative implication of TKI on cancer associated fibroblast was further demonstrated through co-culturing of tumour cells with α -SMA-stained tumour stromal cells, in which significant increase of stromal cell while decline of tumour cell growth following gefitinib treatment. This phenomenon postulates the intrinsic resistance of cancer-associated stromal cells to gefitinib [40]. Recent study analysing the tumour epithelium and stroma in cholangiocarcinoma observed the association of the tumour stroma mediated gene signature and poor patient prognosis. Interestingly, tumour epithelium was associated with HER2 deregulation and aberrant expressions of EGFR and HGFR followed by upregulation of inflammatory cytokines in stroma regions. Treatment with lapatinib, the inhibitor targeted on HER2 and EGFR reprogrammed the tumour microenvironment which favoured of cholangiocarcinoma cell growth suppression [36]. The spleen tyrosine kinase (SYK) has been identified as the B-cell receptor and inhibition of SYK

critically attenuated B cells maturation and survival [41]. Besides targeting on BCR signalling, SYK inhibitor R406 restricted secretion of adhesion factors CXCL12 and VCAM-1 as well as Mcl-1, the chemo resistant factor by stromal cells, which in turn resulted in resistant chronic lymphocytic leukemic cell death [42]. By silencing the receptor tyrosine kinases expressed on fibroblast cells such as FAK and VEGFR, PF562271 [43] and regorafenib treatment [44] reduced populations of fibroblast and interrupting the interaction of tumour cells with stromal cells, resulted in cancer regression. Tumour cells was shown to produce a pro-inflammatory factor, leukaemia inhibitor factor (LIF) upon TGF β exposure and further promoted JAK-mediated actomyosin contractility in cancer-associated fibroblast and reprogramming of extracellular matrix towards pro-invasive phenotype. Accordingly, JAK2 tyrosine kinase inhibitor, ruxolitinib eradicated the remodeling towards pro-invasive phenotype by fibroblast, suggested the JAK inhibition as ideal target for fibroblast inactivation [45]. The phenomenon has also been documented in cancer cells resistance towards TKIs, where addition of roxulitinib re-sensitized the cancer cells towards TKIs exposure [46].

Fibroblast/stromal cells highly expressed PDGF receptors; production of PDGF ligands by cancerous cells triggered the up-regulation of fibroblast growth factors (FGF) by fibroblast which favours of tumour angiogenesis and proliferation. Notably, bone marrow stromal cells highly express PDGFR β , while minimally detected in cancer cells. Therefore, there are emerging strategies targeting PDGFR or FGF on fibroblast in regressing tumour cell growth. For instances, inhibition of PDGFR by imatinib declined the secretion of FGF-2 and FGF-7 by cancer associated fibroblast resulted in reduced tumour progression [47]; combination treatment of nilotinib, PDGFR inhibitor and everolimus, MTOR inhibitor reduced fibroblast and cancer growth [48]; sunitinib treatment abrogated the bone marrow stromal cells growth and adhesion components that facilitate their interactions to tumour cells, thereby interrupting the tumour cells homing and colonization to bone [49]; dovitinib, the multikinase inhibitor attenuated the cross-talk between stromal and epithelial cells mediated by FGFR and regressed prostate cancer growth and bone metastasis [50]. A similar observation was found in primary colonic fibroblast, in which sunitinib attenuated its growth via PDGFR β signalling [51]. On the other hand, FGF2 from tumour microenvironment also have shown to confer resistance of cancer cells towards imatinib; addition of ponatinib, the multikinase inhibitor of BCR-ABL and FGFR re-sensitized the cancer cells [52]. Similar observation of reversing the cancer cell resistance was obtained by addition of PD-173074, FGFR inhibitor to lapanitib treatment [53].

The acquired resistance of tumour cells towards TKI has been frequently reported [46, 54]. The mechanism involved in drug resistance can be diverse, ranging from cell extrinsic to intrinsic conditions. One of the well-known mechanisms inducing resistance is activation/phosphorylation of Met, the proto-oncogene on cancer cells [55]. Apart from acquiring the resistance upon constitutive Met activation in cancer cells, current findings suggested that ligand dependent paracrine receptor activation from TME components initiates the tyrosine kinase inhibitor resistance. Co-culturing of hepatocyte growth factor (HGF), the Met ligand-secreting fibroblast and cancer cells declined cancer cell sensitivity towards TKI treatment [56]. HGF has been shown to highly express in EGFR-TKI resistant lung cancer patients [57]. While co-treatment with another c-MET TKIs, crizotinib [58, 59] or NK4 [60] reversed the drug resistance. Very interestingly, endothelial cells produced EGFR ligand and also initiated the resistance of lung cancer cells towards crizotinib and co-treatment with erlotinib re-sensitized the cells towards crizotinib treatment [61]. By abolishing c-MET activation by fibroblast using DCC2701, a c-MET/TIE-2/VEGFR inhibitor, the cancer growth and migration was subsided. Besides, the HGF produced by normal ovarian fibroblast are much higher than the cancer-associated fibroblast, suggested normal neighbouring fibroblast in supporting cancer cell progression [62]. Another recent finding confirmed the paradoxical activation of cancer associated fibroblast conferred melanoma resistance towards BRAF inhibition, as observed from (a) reduced ERK/MAP kinase activity in reduced stromal density region, (b) co-culture of melanoma cells with fibroblast declined drug-induced apoptosis on melanoma cells, (c) BRAF inhibitor augmented matrix remodelling ability of fibroblast. Incorporation of FAK, Src or PDGFR inhibitors re-sensitized melanoma cells towards BRAF inhibitor in the co-culture system of tumour and stromal cells via re-activation of ERK. Collectively, the findings suggest aiming at the receptor ligand of cancer-associated stromal cells may be ideal to overcome the drug resistance. Disruption of either axis on cancer associated fibroblast with tyrosine kinase inhibitors modulate the cross-talk between tumour and stromal cells, and further augmented the anti-tumour effect [63].

Stem cells

Apart from endothelial cells and fibroblast, mesenchymal stem cells are also able to secrete EGF and express EGF receptor. Suppressing EGF receptor using AG1478 therefore suppressed mesenchymal stem cells mediated mammosphere formation [64]. Yoshida et al. proposed the phenomenon of continuous EGFR activation in EpCAM highly expressed cancer stem like cells while

gefitinib treatment declined the population viability [65]. The same also reported by Lacerda et al. in which erlotinib treatment decreased expression of EGFR in mesenchymal stem cells and declined tumour metastasis [66]. In addition to EGFR, cancer stem cells have also been shown to be responsive to other TKI treatment which further triggered cancer cell death. For instances, treatment of sunitinib declined ALDH+ cancer stem like cell population and re-sensitized the cells to radiation treatment [67]; addition of dasatinib declined the mesothelial-like cell proliferation and thereby attenuated fibrillar formation in metastatic cancer cells [68]; imatinib abrogated cytokines expression that associated with bone marrow homing and leukemic hematopoietic stem cells survival [69]. Recent finding proposed the reprogramming of non-stem like cancer cells to high resistant cancer stem cells following taxane intervention and the transitions was associated with the activation of Src family kinase/Hck signalling. Notably, inhibition of SFK/Hck using RK20449 or dasatinib declined the cancer stem-like populations and allowed temporal chemotherapeutic action [70]. This phenomenon suggested targeting at SFK/Hck signalling with TKI enhanced the tolerance of cancer cells towards chemotherapeutic agents. The phenomenon also has been documented in co-culture of breast cancer cells with bone mesenchymal stem cells, where treatment of saracatinib, the SFK/Hck inhibitor on mesenchymal stem cells blunted the cancer cell migration [71]. Figure 1 summarized the TKIs that targeted on mesenchymal originated cells for subsequent cancer cell regression.

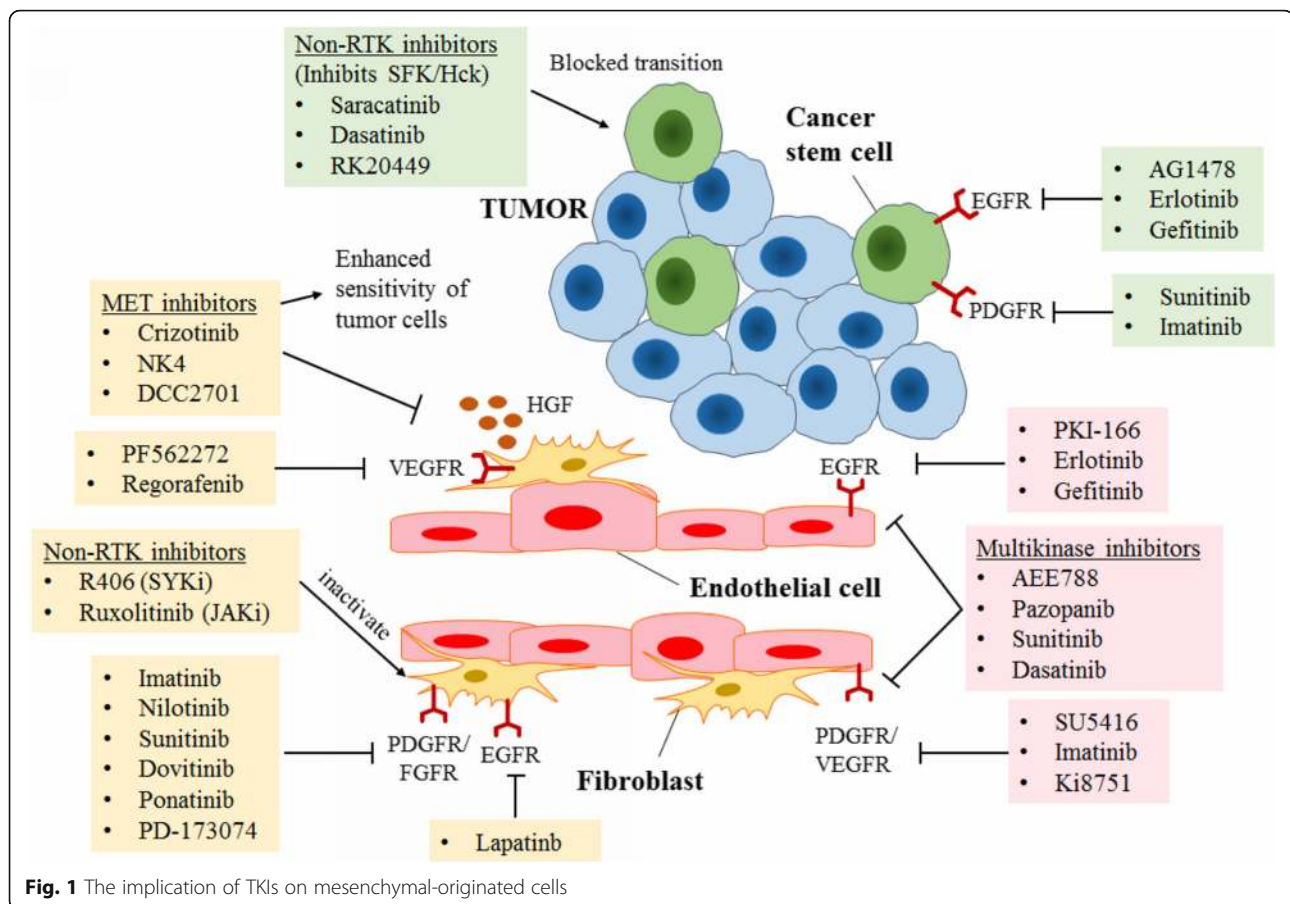
Targeting the haematopoietic cells

Precursor cells

Early findings postulated that the osteoblast, bone marrow precursor cells in bone marrow microenvironment tend to produce substances for instance HGF which further promotes cancer cell proliferation and migratory activities. Intervention of NK4, HGF inhibitor or imatinib mesylate declined the cancer cells functions in the presence of osteoblast [72, 73]. As a Src/Abl kinase inhibitor, dasatinib was known to inhibit the SFK kinase activity and the downstream molecule MMP9 [74]. Apart from restricting the migration of cancer cells, dasatinib also declined the infiltration of MMP9+ myeloid cells and limited their motility through down-regulation of MMP9 [75]. Inhibition of STAT3 using JAK inhibitor AZD1480 also found to decline tumour-associated myeloid population and inhibit myeloid infiltration to tumour site [76].

Immunosuppressive cells

The immunosuppressive environment, which primarily composed of myeloid-derived suppressor cells (MDSCs), regulatory T lymphocytes (Treg) and tumour associated

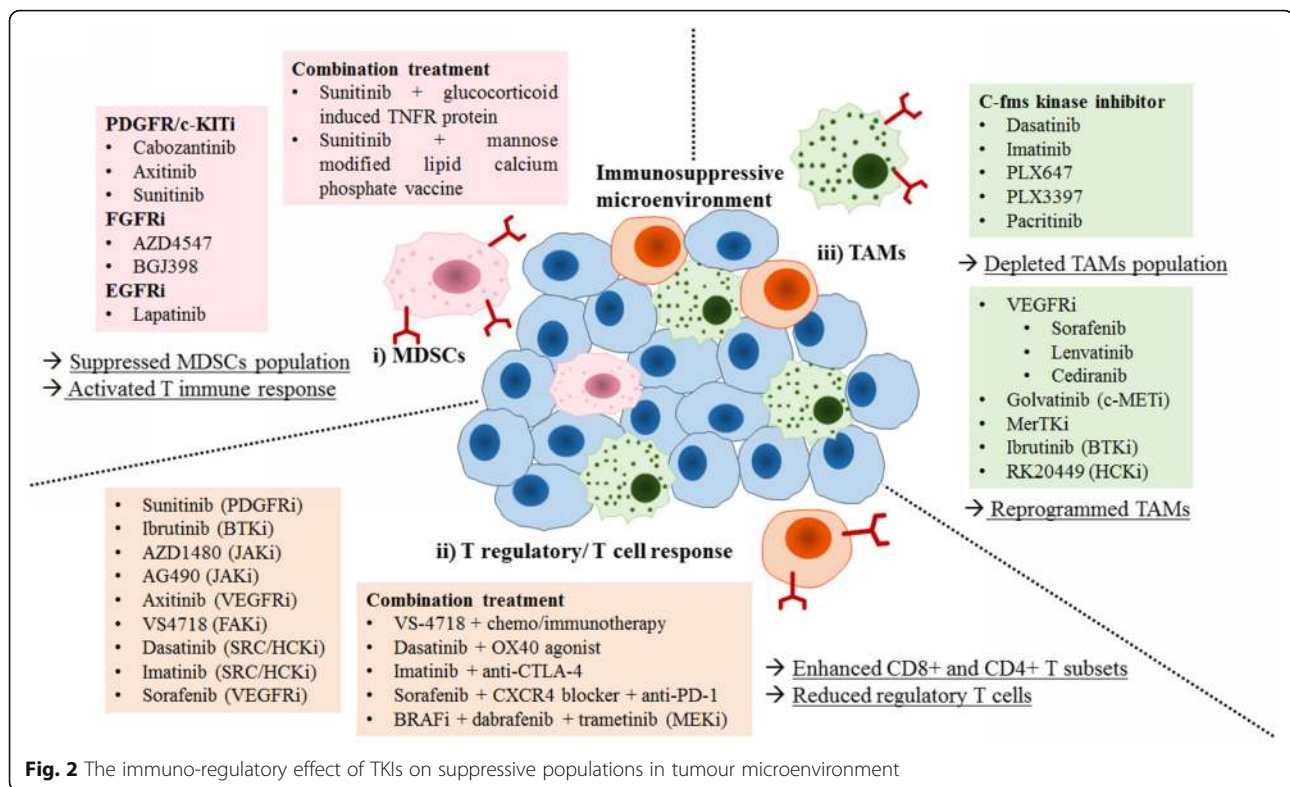


macrophages, promoted escape of tumour cells from immune surveillance [77]. Accumulating therapeutic strategies aimed at immune-suppressive cells in tumour microenvironment to reduce immune tolerance. Evidences have emerged that TKIs switched the immunosuppressive environment and promoted the anti-tumour immunity (Fig. 2).

Myeloid-derived suppressor cells, MDSCs

Myeloid-derived suppressor cells MDSCs, the bone marrow derived heterogenous suppressor cells, has been shown to positively correlate to cancer progression. In advanced stage of cancer patients, tumour may induce expansion of MDSCs and in turn resulted in suppressive action of innate and adaptive immune response by high populations of MDSCs in tumour microenvironment [78]. Sunitinib treatment has found to decline MDSCs populations and MDSCs mediated regulatory T cell development in tumour tissues. Further mechanism study showed sunitinib modulated the co-stimulatory molecule and secretive factors profiles as well as composition of tumour infiltrating leukocytes favouring of T cell activation. Observing sunitinib co-operatively targeting on VEGFR2, c-kit receptor, PDGFR as well as Flt3, further

investigation postulated that c-KIT is responsible on the suppressive action of sunitinib on MDSC expansion and survival [79]. Another study by Xin et al. also demonstrated the suppression of MDSCs by sunitinib via STAT3 inhibition [80], suggested the specific blockade of c-KIT or VEGFR1–3 using TKIs on MDSCs may be effective in producing a permissive immune-competent tumour microenvironment. Thus, it is not surprised observing accumulating studies on TKIs targeted at VEGFR and c-KIT in reversing the suppressive microenvironment. For instance, use of axitinib, the VEGFR inhibitor declined MDSCs while induced CD8 T cell infiltration to melanoma [81, 82]; cabozantinib, a multikinase inhibitor perturbed the immune cells subset and reduced MDSCs and regulatory T cells in spleen [83]. Moreover, the capability of sunitinib in disrupting MDSCs/Treg subsets in tumour microenvironment may enhance the response of tumour cells towards immunostimulating therapies such as vaccines or antibodies intervention. For instances, the synergistic use of sunitinib with glucocorticoid induced TNFR related protein (GITR) increased infiltration of CD8(+) T and natural killer populations followed by repolarization of macrophages towards immuno-competent M1 phenotype that



favours of tumour regression [84]. Similar observation also obtained on combination treatment of encapsulated sunitinib with mannose-modified lipid calcium phosphate vaccine on melanoma, in which the immune response was shifted towards Th1 phenotype resulted in tumour apoptosis [85]. Despite the potent effect of VEGFR/c-KIT targeted TKIs on MDSCs as reported, the resistance of MDSCs towards sunitinib treatment has also been reported and the intra-tumoral survival of MDSCs was associated with STAT5 activation and GM-CSF upregulation [86, 87]. It is supported by another observation that prolonged use of sunitinib in orthotopic glioma bearing mice triggered restoration of macrophages and Gr1 (+) MDSCs thereby promoted aggressive phenotype of tumour cells [88]. These findings suggested that single use of sunitinib may not be sufficient in attenuating development of cancer resistance and secondary therapy is needed to support the sustained immunosuppression induced by VEGFR/c-KIT targeted TKIs.

In addition to VEGFR/c-KIT as therapeutic target of MDSCs, the use of AZD4547, the FGFR inhibitor has also been proposed as a target of MDSCs action. AZD4547 reduced breast cancer proliferation and migration through declining the MDSCs population while enhancing T populations [89]. Consistent with the finding, BGJ398, another FGFR inhibitor was found to inhibit MDSCs population and tumour vasculature. Although

tumour relapse was observed after 4 months, the combination uses of EGFR inhibitor, lapatinib with BGJ398 significantly deferred the tumour reversion [90]. Another prominent recent finding demonstrated that enhancement of MDSCs population in BRAF inhibitor-resistant tumour microenvironment which initially reduced after BRAF inhibitor treatment. The phenomenon was not observed in regulatory T cells, which postulated that the tumour resistance was contributed by restored MDSCs recruitment via CCL2/CCR2 regulation. Further study, nevertheless, showed solely MDSCs depletion could not re-sensitize tumour cells towards BRAF inhibitor treatment, while combination treatment with immunotherapies anti-CTLA-4 and anti PD-1 elicited tumour cells to immunotherapy-triggered cancer cell death [91].

T lymphocytes

Accumulating studies have related the cross-talk between MDSCs and regulatory T subset, generally, MDSCs recruited and supported the activation of T regulatory populations [92]. Thus, it is not surprising to observe MDSCs reduction by TKIs further led to regulatory T subset suppression. Nonetheless, as a matter of fact, immune microenvironment is dynamic and the responses are affected by extensive cross-talk between immune populations at different levels. The direct target of TKIs on T populations has also been widely described. Using adoptive transfer method, Kujawki et al. showed

that sunitinib treatment with adoptively transferred T populations declined the shift of FoxP3 (-) T to regulatory T phenotype as well as increased CD8 (+) cytotoxic T infiltration. The phenomenon is explained as sunitinib-mediated STAT3 inactivation in T populations further enhanced the STAT3-deficient T cells infiltration [93]. Ibrutinib, an inhibitor of Bruton's tyrosine kinase which is originally designed for inhibition of B cell malignancy [94], was found to reduce CD4 (+) T population and PD-1 expression on chronic lymphocytic leukemic patients. Further study observed dislodge of macrophages with leukemic cells as well as reduced chemo-attractant CXCL13 following ibrutinib treatment [95]. Concomitant use of ibrutinib with toll like receptor ligand also enhanced the antigen presenting property of cancer cells for T cell recognition [96]. Moreover, the JAK inhibitor, AZD1480 and AG490 has been described to reduce suppressive T populations while augment anti-tumour CD8 (+) T, M1 macrophages and natural killer populations [97, 98]. Likewise, the VEGFR inhibitor, axitinib also has been shown to increase CD4 (+) and CD8 (+) T population, while progressive patients showed increase regulatory T subset and PD-1 expression following axitinib treatment [99]. This further suggests acquiring resistance of immune cells to axitinib treatment upon disease progression. One mechanism involves the focal adhesion kinase (FAK) has been shown to regulate the transcriptional activation of chemokine and cytokines that further facilitates T cells response. The authors showed FAK-mediated paracrine secretion of CCL5 promoted regulatory T population while reduced CD8 (+) T subsets. Inhibition of FAK kinase using VS-4718 markedly augmented CD4 (+) and CD8 (+) populations while declined regulatory T subsets [100]. Another report implicated VS-4718 treatment augmented the sensitivity of tumour cells towards chemo- and immuno-therapies, primarily through declining the immunosuppressive M2 macrophages, GR1 (+) granulocytes and regulatory T populations [101].

Dasatinib, a multikinase inhibitor, reduced T cellular proliferation, its associated cytokine production and cellular response [102, 103]. The inhibitory effect of dasatinib on T cells is mediated by LCK inactivation, the member of Src family that regulates T-cell receptor TCR signalling. Consistent with the findings, recent study specifically showed dasatinib treatment reduced regulatory T populations while enhanced CD8 (+) anti-tumour T response. Co-stimulation of T response using OX40 agonist induced synergistic tumour inhibitory effect compared with dasatinib or anti-OX40 alone [104]. Consistently, imatinib mesylate, another inhibitor of Src family kinases enhanced CD8 (+) T population and cellular proliferation while declined regulatory T subset. Co-administration of imatinib with anti-CTLA-4, the T cell

modulator showed greater tumour regression compared with single treatment [105]. Similar mechanism as dasatinib and imatinib has also been documented by sorafenib treatment, in which sorafenib also impaired T cells activation through LCK inactivation on T cells [106, 107]. In this regard, sorafenib treatment increased CD4 (+), CD8 (+) T population while reduced PD1 (+) CD8 (+) T as well as declined the regulatory T cellular function and proliferation [108, 109]. In line with these observations, clinical investigation on 45 sorafenib-receiving HCC patients showed reduced Th2 and regulatory T populations after high dose treatment of sorafenib (400 mg and 800 mg/day) with mean plasma concentration of 3-6 mg/L, suggested the immune-modulatory effect of sorafenib [110]. However, another contradictory finding in advanced hepatocellular carcinoma HCC postulated the increase of immunosuppressive T regulatory, M2 macrophages as well as intra-tumoral PD-L1 expression following sorafenib treatment, which is mediated by enhanced hypoxia in tumour microenvironment. Combination treatment of sorafenib with CXCR4 blocker and anti-PD-1 reprogrammed the immunosuppressive condition to anti-tumour phenotype and further induced HCC regression [111]. In support of this notion, Cabrera et al. postulated the dose-dependency of sorafenib on immuno-regulation, in which reduced IL2 secretion by CD4 (+) T cells while increased regulatory T suppressive activity above 3 μ M of sorafenib treatment [112]. These findings further suggested the existence of dose response of TKIs on immune-modulation and the optimal dose sought may offer the modest clinical benefit for patients.

On the other hand, many small molecules inhibitors may acquire resistance upon disease progression whereby single treatment may not sufficient in altering the immunosuppressive tumour microenvironment. The VEGFR inhibitor, axitinib has been shown to increase CD4 (+) and CD8 (+) T population, while progressive patients showed increase regulatory T subset and PD-1 expression following axitinib treatment [99]. Likewise, BRAF inhibition initially enhanced CD8 (+) T infiltration and reduced immunosuppressive factors in melanoma patients, however, the CD8 (+) T population gradually decreased along with disease progression [113]. Combination treatment of BRAF inhibitor and MEK inhibitor, dabrafenib and trametinib re-established the T cells population, suggesting combination treatment may be beneficial for the rebound immune profile in progressed cancer. This is in accordance with the notion that MEK activation exerts cancer cell resistance while inclusion of MEK inhibitor may overcome it [114].

Tumour associated macrophages, TAMs

Much of the past and current efforts in eliminating the tumour associated macrophages (TAMs) have focussed

on blocking TAMs survival or recruitment, inhibiting TAMs function and re-polarizing the TAMs phenotype that favour tumour regression. Typically, the *c-fms* kinase, or known as CSF1 receptor is a ligand-activated kinase that modulate monocytes and macrophage survival and proliferation [115]. The potent 'off-target' inhibitors of FMS receptor, dasatinib and imatinib have been shown to inhibit the growth of recruitment and growth of macrophages [116, 117]. Notably, imatinib treatment modestly depleted macrophage populations after 4 weeks of treatment in gastrointestinal stromal tumour. It was followed by cytokine profile shifting towards anti-tumour phenotype via activation of CCAAT/enhancer binding protein β . The TAMs phenotype re-shifted to tumour-promoting-like upon cancer cells acquired resistance towards imatinib [118]. PLX647, a FLT3 inhibitor also showed 35-fold above selectivity against its enzymatic homologs FMS at 1 μ M [119]. Consistently, PLX647 treatment significantly suppressed macrophage activation and proliferation as governed by FMS. The same also observed on its counterpart compound PLX3397, in which it enhanced the sensitivity of cancer cells towards chemotherapeutic treatment through declining TAMs infiltration to tumor microenvironment [120]. Observing the homologue of CSF1R and FLT3, pacritinib, the JAK2/FLT3 inhibitor, has also been shown to interfere CSF-1R signaling. Treatment of pacritinib on nurse-like cell, the monocyte-like cells in chronic lymphocytic leukemia CLL led to nurse-like cell death and CLL regression [121]. Although CSF1R-targeted TKIs may potentially deplete macrophages, it is arguable that systemic blockade of all macrophage populations may be detrimental.

Therefore, apart from macrophage depletion, therapeutic strategies in re-programming TAMs immunosuppressive phenotype towards anti-tumour profile by TKIs intervention have also emerged as potent TAMs-targeted cancer therapy. For instances, sorafenib treatment stimulated IL12, IL18 and IL6 production in TAMs after LPS priming, which further supported natural killer cells function [122]. Tie2 receptor has been shown to highly express on monocytes/macrophages and its paracrine secretion supports tumour neovascularization [123]. Study by Nakazawa et al. postulated that the *c-MET* inhibitor golvatinib, which also interfere the Ang-Tie2 and EphB4-EphrinB2 signalling, reprogrammed TAMs phenotype towards M1 anti-tumour-like. The golvatinib treatment also reduced macrophage infiltration into tumour sites via Ang-Tie2 blockade [124]. Besides, the authors also demonstrated combination treatment of golvatinib with lenvatinib, the VEGF inhibitor sensitized cancer cells towards lenvatinib treatment, further justified the previous study that Ang2 inhibition reversed the cancer resistance of VEGF inhibitor [125]. Likewise, the combination treatment of

cediranib, pan-VEGFR inhibitor with anti-Ang-2 altered TAMs phenotypes, further led to vessel normalization in glioblastoma [126]. As one of the members in the family of TAMs associated receptor tyrosine kinases (RTK), MerTK has been previously shown to mediate the primary functions of macrophages -to engulf and phagocytize bacteria [127]. In this regard, macrophages in human glioblastoma highly expressed MerTK while UNC20205, the MerTK inhibitor treatment declined M2 macrophage population in glioma associated microenvironment [128]. Likewise, MerTK inhibitor also decreased M2 macrophage associated TGF β secretion, thereby led to reduced tumour metastasis [129].

Accumulating studies postulate the BTK was activated/phosphorylated upon Toll like receptors (TLR) stimulation [130], and activation of BTK was associated with TLR3-associated cytokine production in macrophages [131]. Treatment of ibrutinib declined chemokines CXCL12, CXCL13, CCL19 and VEGF production by TAMs, which in turn resulted in declined invasion and migration of lymphoid cells [132]. Likewise, ibrutinib also enhanced the anti-tumour phenotype of TAMs in chronic lymphocytic leukemia [133]. Recent study also postulated that the hematopoietic cell kinase (HCK), which belongs to the Src kinase family is constitutively expressed on myeloid cells and associated with tumour initiation and survival. Besides, the TAMs polarization towards M2 phenotype is regulated by HCK activation, while pharmacological inhibition of HCK using RK20449 declined M2-like macrophage accumulation [134]. The phenomenon is consistent with previous finding that HCK primarily mediated M2-like immunosuppressive gene expression in macrophages/monocytes [135]. The emergence of this tyrosine kinase for macrophage polarization supports the potential use of SFK-inhibitor such as dasatinib and imatinib, to reprogram the immunosuppressive macrophages to anti-tumour phenotype through blunting the HCK activity of TAMs.

Others

Recruitment of mast cells to tumour microenvironment has been shown to promote pancreatic cancer cell survival and inhibition of mast cell degranulation led to cancer cell death. Intervention of PCI-32765, the BTK inhibitor, declined mast cells degranulation and cancer cell growth [136]. The same phenomenon also documented in ibrutinib-treated pancreatic tumour, whereby ibrutinib treatment diminished the mast cell associated fibrosis [137]. Despite the effect of TKIs on neutrophils rarely been described, Patnaik et al. showed cabozantinib, multi-kinase inhibitor treatment induced neutrophils infiltration into prostate tumour sites. Further investigation postulated that dying tumour cells produced CXCL12 and HMGB1 to recruit and activate

neutrophils for following cancer eradication after cabozantinib intervention [138].

Targeting the non-cellular components

Extracellular matrix ECM components including fibronectin, laminin and collagen have been shown to determine the fate of cancer cells whether to remain dormant or proliferative [139]. It was demonstrated that collagen or fibronectin accumulation promoted progression of dormant breast cancer cells via β 1-integrin mediated SRC and FAK activation. SFK inhibition with AZD0530 offered transient blockade on proliferation of dormant cancer cells, and only combination of AZD0530 with selumetinib, MEK inhibitor prevented the dormant-to-proliferative shift of cancer cells [140]. Furthermore, the composition of ECM also governs the therapeutic outcome of cancer treatment. Earlier study postulated that small cell lung cancer cells tend to accumulate at ECM rich region, whereby the ECM proteins further protected the cancer cells from chemotherapy. The adhesion of cancer cells to fibronectin triggered activation of protein tyrosine kinase and intervention with tyrphostin-25, a competitive EGFR tyrosine kinase inhibitor abolished the effect [141]. The similar phenomenon has also been documented by another two potent TKIs genistein and tyrophostin AG-1478 [142].

Perspectives on combination treatment of TKIs and immunotherapy

It has been proposed in various types of human cancers that immunotherapeutic regimen could offer favourable outcome, though large-scale randomized, placebo-controlled trials shall be carried out prior to its application. Given the accumulating reports on acquired resistance of TKIs, the use of TKIs alone as first-line treatment seems challenging in spite of its better prognosis. TKIs treatment often gain rapid but not durable tumour response, which result in early improvement of survival curve of cancer patients without clear beneficial effect on the overall survival. The effect of TKIs could be strategically complemented by immunotherapy, which induces a low percentage but highly durable tumour response [143]. This notion has been partially supported by pre-clinical observation in mouse model, which showed that an ALK vaccine improved tumour relapses after TKI suspension [144]. The idea combining TKIs with immunotherapy raised in early 2010s and some efforts, in both pre-clinical and clinical extent, have been made to investigate its beneficial effect in cancer treatment [145]. Although most of the studies are still on-going, progress report in recent conference proceedings have suggested the prominent potential of this brilliant combination. For instance, McDermott et al. reported that combination of Atezolizumab (anti-PD-L1)

with sunitinib (VEGFR inhibitor) resulted in encouraging anti-tumour effect than TKIs alone in metastatic renal cell carcinoma (mRCC), and a large scale phase III trial is highly suggested [146]. On-going studies from Ribas group suggested combination of pembrolizumab (anti-PD1) with dabrafenib (BRAF inhibitor) and trametinib (MEK inhibitor) for phase II study in BRAF-mutant melanoma patients after manageable toxic profile of this combination has been observed [147]. Mechanistically, the action of combination treatment may be in sequential or parallel. For instances, sequential use of sorafenib followed by sunitinib improved overall survival of the metastatic renal cell carcinoma patients, suggesting the sequential action of this combination [148]. However, given the regulation of TKIs on tumour micro-environment as we recognized in this review, it has been expected that the combination treatment would deliver a parallel action, that is, TKIs and immunotherapy synergize each other. It was observed that combination of anti-angiogenic TKIs sorafenib/sunitinib with anti-tumour rMVA-CEA-TRICOM vaccine, could synergistically increase the CD8+ tumour infiltrating lymphocytes and intratumoral CD11b+ cells, which is hardly observed in TKIs or vaccine treatment alone. This is partially related to the regulation of TKIs alone on the phenotypes of immunosuppressive MDSCs and TAMs [149]. Combination of TKIs with FLT3-directed immunotherapy enhanced its therapeutic outcome on acute myeloid leukaemia, which is probably due to the induced expression and localization of FLT3 on the cell surface [150]. Efficacy and mechanism of action of combination treatment using TKIs and immunotherapy are expected to be under more extensive inspection.

Furthermore, safety of the combination use is still contradicting and unclear. Some studies have reported the increased risk of adverse reaction after combination therapy, including higher incidence of grade 3/4 liver enzyme elevation (40–70%) and high incidence of interstitial lung disease (38%) [151]. A lot of recent efforts were thus made to justify the safety use of combination treatment. A phase I study on the safety of combination treatment of cobimetinib (MEK inhibitor) and atezolizumab (anti-PD1) showed that the well tolerance of colorectal cancer patients at the maximum administered doses [152]. Combination of atezolizumab (anti-PD1) with vemurafenib (BRAF inhibitor) and cobimetinib (MEK inhibitor) induced rash and elevated liver enzymes in patents with BRAF-mutant melanoma, however, the adverse reaction was manageable by a run-in period of vemurafenib and cobimetinib [153]. However, unsuitability of combination treatment between immunotherapy and TKIs was also reported. For instances, combination of pazopanib (TKIs) and pembrolizumab (anti-PD1) resulted in significant hepatotoxicity in advanced RCC

patients. While sequential regimen showed improved efficacy yet limited tolerability [154]. Interestingly, it was somewhat suggested that combination treatment may improve the adverse reaction, which may be probably due to the dose-reduction effect in TKIs. Bhatia et al. reported a favoured outcome in clinical trials using combination of IL21 with sorafenib to treat mRCC. The combination use of IL21 resulted in appropriate dose-reduction in sorafenib associated with improved adverse reaction [155]. These pilot findings suggested the safety of combinatory treatment of immunotherapy and TKIs on cancer patients warrant continued exploration for clinical benefit.

Overcoming TKIs resistance through modulating tumor microenvironment

The development of acquired resistance remain as the major clinical challenge to TKIs treatment, despite TKIs are still the first line therapeutic regimen for many malignancies. For instances, non-small cell lung cancer patients who initially well responded to EGFR inhibitors, erlotinib or gefitinib had host adaptive response and tumor growth within 6–12 months [156, 157]. Majority of the first line TKIs aiming at the intrinsic function of tumor cells, while neglecting that the susceptibility of tumor cells may shift in response to the alteration of microenvironment. In fact, upon TKIs perturbation, the tumor microenvironment compartment tends to secrete the extrinsic factors such as cytokines, hormones, or growth factors in sensitizing or de-sensitizing the response of cancer cells. This notion was further supported by pre-clinical observations that the paracrine secretions of various growth factors by neighboring fibroblast such as hepatocyte growth factors (HGF) and neuregulin 1 (NRG1) further promoted cancer cell resistance to TKIs [60, 158]. While re-sensitization of cancer cells to TKIs is often achieved by either suppressing the secretive factors by TME or antagonizing the receptors on cancer cells. It was observed that co-administration of EGFR inhibitors with apatinib, a highly selective VEGFR2 inhibitor reduced the expression of VEGF in TME, which further delayed tumor growth in mice and prolonged disease-free survival in cancer patients [159]. The fibroblast and endothelial cells produced HGF and EGFR ligands reduced the cancer cell response to crizotinib, a dual inhibitor of ALK and Met. While co-administration of EGFR inhibitors enhanced the susceptibility of cancer cells to crizotinib [61]. These attempts suggest that manipulating TME by repressing pro-tumoral secretive factors or blocking receptors on cancer cells may be beneficial in overcoming TKIs resistance.

Also, it is believed that cancer cells develop an immunosuppressive microenvironment for evasion from

immune surveillance upon TKIs intervention. Although the detailed mechanisms of acquired immune evasion is not yet understood, previous studies suggested that the inducible expression of immune checkpoints PD-1/PD-L1 may be involved. Notably, the elevated expression of PD-L1 was observed in EGFR-resistant tumor biopsy compared to pre-treated tumor samples. This suggests the activation of immune checkpoint may hinder the treatment efficacy of TKIs to cancer patients [160]. Furthermore, co-treatment of immune checkpoint inhibitor, nivolumab in lung cancer patients after disease progression with TKIs showed improved progression-free survival and the enhanced progression-free survival rate was in proportion to PD-L1 expression [161]. Similar observation was obtained from patients receiving ALK inhibitors, whereby anti-PD-1 immunotherapy restored the efficacy of ALK-targeted therapy [144]. Therefore, the treatment strategies aim at normalizing or reprogramming the immunosuppressive cancer environment may be promising in reversing TKIs resistance and achieve a favorable outcome of TKIs-associated cancer therapy.

Our experience: Immuno-modulatory complementary therapy potentiates TKIs treatment

With recognition of the actions of TKIs on TME, our previous studies have also suggested the beneficial role of complementing TKIs with immune-modulatory therapy. Complementary treatment using PHY906, a composite herbal formula that had potential to increase the therapeutic index of cancer treatments in multiple clinical trials, was recently identified to modulate TME in sorafenib-treated hepatocellular carcinoma (HCC). This immune-modulatory effect of PHY906 improved the anti-tumour TME signature and thereby enhanced the efficacy of sorafenib [162]. An active component isolated from PHY906, named baicalin, has evidenced to contribute to this immuno-modulatory action by specifically targeting on autophagic pathway in TAMs [163]. These observations combined with our study on other natural molecules suggest the potential of using immuno-modulatory complementary therapy [164] in combination with TKIs as emerging cancer therapy.

Conclusion

In past decade, tyrosine kinase inhibitors targeting at tumour cells represent a promising therapeutic agent for cancer patients; yet, the use of TKIs in modulating tumour microenvironment is still in its infancy. The recent advances we summarized here emphasize the potential use of TKIs for re-educating or normalizing the dysregulated tumour microenvironment for cancer treatment. An understanding of the modulatory effect of TKIs on tumour microenvironment could expedite the

maximal use of TKIs on cancer therapy as well as repositions the existing TKIs as combination cancer treatment. Furthermore, phosphorylated status of target protein could also be determined by protein phosphatase, which belongs to another group of protein involved in microenvironment status of tumour cells, is worthy for further exploration. Much more efforts, either pre-clinically or clinically, is therefore anticipated in order to maximize the efficacy and safety for patients.

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Authors' contributions

YF and YCC conceived and designed the study; HYT and NW retrieved data and wrote the manuscript. All authors discussed and revised the manuscript and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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