Pharm Res (2012) 29:621–636
DOI 10.1007/s11095-011-0645-9

EXPERT REVIEW

Novel Anti-cancer Compounds for Developing Combinatorial Therapies to Target Anoikis-Resistant Tumors

Lokesh Nagaprashantha • Neha Vartak • Sangeeta Awasthi • Sanjay Awasthi • Sharad S. Singhal

Received: 12 October 2011 / Accepted: 5 December 2011 / Published online: 28 December 2011

ABSTRACT Anoikis, a cell death pathway induced by loss of normal cell-matrix attachment or upon adhesion to a non-native matrix, ensures the balance between proliferative potential of normal cells and maintenance of tissue integrity. Thereby, anoikis serves as a potential molecular barrier against oncogenic transformation of normal cells. Cancer cells acquire anoikis resistance for survival and distant metastatic progression. During the acquisition of anoikis resistance, tumors modulate multiple cell signaling parameters through changes in the expression of up-stream receptors and by dynamically calibrating the dependency on down-stream signaling cascades. Many compounds that target the tumor-acquired switches in integrins, tumor antigens, growth factors, metabolic pathways, oxidative and osmotic-stress signaling are in various phases of pre-clinical and clinical development. Combinatorial approaches maximize the therapeutic efficacy and minimize the activation of alternate signaling pathways, which will otherwise contribute to drug resistance. In this regard, an integrated analysis of the mechanisms of action of potential drugs and lead compounds that can target significant nodes of anoikis signaling networks will provide a rational frame-work for further development and clinical use of respective agents, by formulating more effective combinatorial therapies, in patients with distinct drug-sensitivity profiles.

L. Nagaprashantha · S. Awasthi · S. Awasthi · S. S. Singhal (✉)
Department of Diabetes and Metabolic Diseases Research
Beckman Research Institute, City of Hope, National Medical Center
Duarte, California 91010, USA
e-mail: ssinghal@coh.org

N. Vartak
Department of Molecular Biology and Immunology
University of North Texas Health Science Center
Fort Worth, Texas 76107, USA

KEY WORDS anoikis · drug-resistance · metastases · network-targeted cancer therapeutics

INTRODUCTION

Anoikis: A Conceptual Framework

Normal cells are directed to programmed proliferation, and are constantly monitored to organize themselves into a distinct cyto-architecture corresponding to the respective tissue of origin. The signals relayed from cell surface receptors like integrins are processed by highly interacting intracellular molecular complexes, which regulate the transduction of signals generated from sensing of the extracellular matrix (ECM) (1). The down-stream cell signaling pathways form highly interacting channels for delivering the inputs from cell-matrix surveillance to reprogram the cellular proliferative, apoptotic, metabolic, oxidative, osmotic and transcriptional networks in response to dynamic changes in the nature of cell-matrix contacts (2). Anoikis (Greek term meaning "homelessness" or "loss of home") is a process of cell death initiated consequent to loss of cell-matrix attachment or upon establishment of inappropriate cell-matrix contacts. Hence, anoikis serves as an inherent intracellular barrier against the formation and survival of potentially oncogenic clones originating within normal tissues and organs (3).

Molecular Events Regulating Anoikis Resistance in Tumors

The incidence and progression of cancers is regulated by the interplay of multiple signaling cascades in a spatially and temporally regulated manner, depending on the origin of

tumors, site of colonization and the stage of disease. The tumors initiate changes in the expression of integrins, growth factor receptors, oxidative and osmotic-stress cascades in order to acclimatize to the constantly changing nature of the cell-matrix contacts. Among cell-matrix sensors, integrins serve as master regulators of cell-matrix attachment that relay the signals transduced from respective ECM ligands to intracellular signaling networks (1–3). The integrin family of receptors in vertebrates is comprised of 18 α and 8 β subunits, which on ligation give rise to 24 different types of integrins (4). The expression of integrins has a tissue-specific pattern, which is significantly altered in tumors during acquisition of anoikis resistance and malignant progression. Activation of focal adhesion kinase (FAK) plays a critical role in regulating the integrin-driven relay of cell-matrix surveillance signals into cells. FAK binds to cytoplasmic tails of integrins and undergoes autophosphorylation on the Y^{397} residue to convey cell-matrix adhesion-induced survival signals (5). The SH2 domains of Src family kinases bind to the pFAK (Y^{397}) and further activate FAK by feedback mechanism. Adaptor protein Grb2 binds to pFAK (Y^{925}), which initiates a signaling that activates RAS-RAF-MEK-ERK pathway (6). In effect, FAK overexpression leads to ERK activation followed by phosphorylation and proteosome-dependent degradation of pro-apoptotic Bim (7). FAK also relays its signals through PKB/Akt pathway to increase the phosphorylation-dependent sequestration of pro-apoptotic protein Bad with 14-3-3 protein, which eventually leads to anoikis resistance (8). Silencing of FAK by siRNA enhances anoikis in anoikis-resistant pancreatic cancer cells (9). Hence, changes in the expression and signaling pattern of integrins play a significant role in the survival and progression of anoikis-resistant tumors.

During acquisition of anoikis resistance, many of the epithelial tumors acquire mesenchymal phenotype, which is commonly referred to as "epithelial-mesenchymal-transition" (EMT) (10). EMT is initiated by a coordinated reprogramming of cellular genetic and protein signaling networks. E-cadherin is considered as a marker and essential signaling factor in maintaining the normal epithelial phenotype of cells (11). Snail is a zinc finger family transcriptional repressor, which is overexpressed in many tumors including high-grade breast tumors (12). Snail binds to the sin-3A corepressor complex containing histone deacytylase, which in turn inactivates chromatin and inhibits the transcription of E-cadherin (13). The onset of EMT also leads to increased expression of specific growth factors like epidermal growth factor (EGF), fibroblast growth factor (FGF) and hepatocyte growth factor (HGF) which increase the activation of down-stream PI3K, MAPK and mTOR survival cascades (10,14,15). The enhanced activation or enabling mutations in the down-stream signaling cascades like oncogenic RAS promote anoikis-resistance by further activating Rac and Rho which eventually leads to increased metastatic potential in tumors (16). EGFR and the signal transduction and activator of transcription 3 (STAT3) cooperatively induce EMT by up regulating the transcription factor twist (17). Thus, the increased expression of transcription factors snail and twist initiate reprogramming of cellular transcription machinery to induce EMT. The molecular changes induced during EMT in turn enhance the ability of cancer cells to acquire anoikis resistance by modulating both the nature of cell-matrix surveillance as well as down-stream cell signaling cascades (18,19).

Anoikis Resistance and Metastatic Potential of Tumors

In a classical breast cancer study revealing the mechanistic link between EMT, anoikis resistance and metastases, the epithelial specific knock-out of E-cadherin and p53 lead to increased formation of invasive and metastatic mammary carcinomas along with induction of anoikis resistance and angiogenesis (20). The increased expression of X-linked inhibitor of apoptosis (XIAP) induces anoikis resistance in circulating metastatic prostate cancer cells that are deprived of normal cell-matrix environment (21). In another study, silencing of carcino-embryonic antigen cell adhesion molecule 6 (CEACAM6), which activates Akt and induces anoikis resistance, lead to not only anoikis sensitization but also inhibition of metastatic potential of pancreatic adenocarcinoma cells (22). In effect, the acquisition of anoikis resistance contributes to the survival of transformed cells with aberrant cell-matrix contacts, invasion of tumor cells into non-native ECM, survival in matrix-deprived conditions while traversing to different organs and metastatic colonization in distant organs with different cyto-architecture (2,3,10,18,19).

Though the current clinical interventions for treating cancers have benefitted from advances in molecular medicine, formulation of effective strategies that match the dynamic and adaptive plasticity of tumors is still a strong focus of contemporary investigations in anti-cancer drug development. The individual genetic profiles and presence of co-morbid diseases in the elderly population, who are commonly predisposed to majority of tumors, represent some of the common factors, which influence the choice of drugs, dosing, considerations regarding toxicities and potential clinical outcomes (23). Recent advances in cancer biology have enabled the characterization and integration of complex genetic and molecular profiles of tumors leading to emergence of combinatorial cancer therapeutics, which is serving as an integrated and more reliable platform to assess the potential lead compounds for further testing of rational drug combinations (24). In this regard, various anti-cancer compounds that target specific signaling nodes regulating

anoikis resistance present precious opportunities for targeting aggressive and metastatic tumors (Fig. 1).

ROLE OF DRUGS TARGETING CELL-MATRIX SURVEILLANCE NETWORKS IN ANOIKIS RESISTANCE

Role of αvβ3 Integrin/B-RAF/MEK Signaling Axis in Targeting Anoikis Resistance of Melanomas

Metastasizing melanomas acquire aberrations in integrin expression and associated down-stream signaling pathways. While invading the dermal matrix, melanoma cells switch from normal αvβ1 to αvβ3 integrin, which facilitates acquisition of anoikis resistance (25). In 66% of melanomas, the substitution of valine by glutamic acid at 600 amino acid of B-RAF (B-RAF^{V600E}) is an important molecular event, which leads to anoikis resistance by continuous activation of MEK/ERK and consequent inhibition of pro-apoptotic Bim (26). Melanomas expressing B-RAF^{V600E} are more susceptible to down-stream MEK inhibition (27).

In the context of alternation of anoikis signaling in melanomas, the characterization of IH 1062 (3, 5-dichlorophenylbiguanide), which can inhibit the αvβ3 integrin is a salient development. IH1062 attenuates FAK phosphorylation and induces anoikis in anoikis-resistant M21 mouse melanoma cells. IH1062 is also effective in decreasing the pulmonary metastases of melanoma cells in mice tail-vein anoikis model (28). Recent FDA approval of vemurafenib (Zelboraf), a novel B-RAF^{V600E}-specific kinase inhibitor, along with a mandatory 4800 BRAF V600 mutation test is another salient development for the clinical management of B-RAF^{V600E}-expressing, un-resectable or metastatic melanomas (29). A MEK inhibitor called AZD6244 has shown some beneficial effects in a subset of melanoma patients with B-RAF^{V600E} mutations in phase II clinical trial (30). Also, a hydrogen sulfate capsule of AZD6244 has been successfully tested in human malignancies. The results from these studies on AZD6244 have shown that the formulation of AZD6244 capsule is tolerable and effective in inducing anti-tumor effects in phase I clinical studies (31). Further trials and combinations focused on αvβ3 integrin/B-RAF^{V600E}/MEK inhibitors would be helpful to target anoikis resistance and metastatic progression of melanomas.

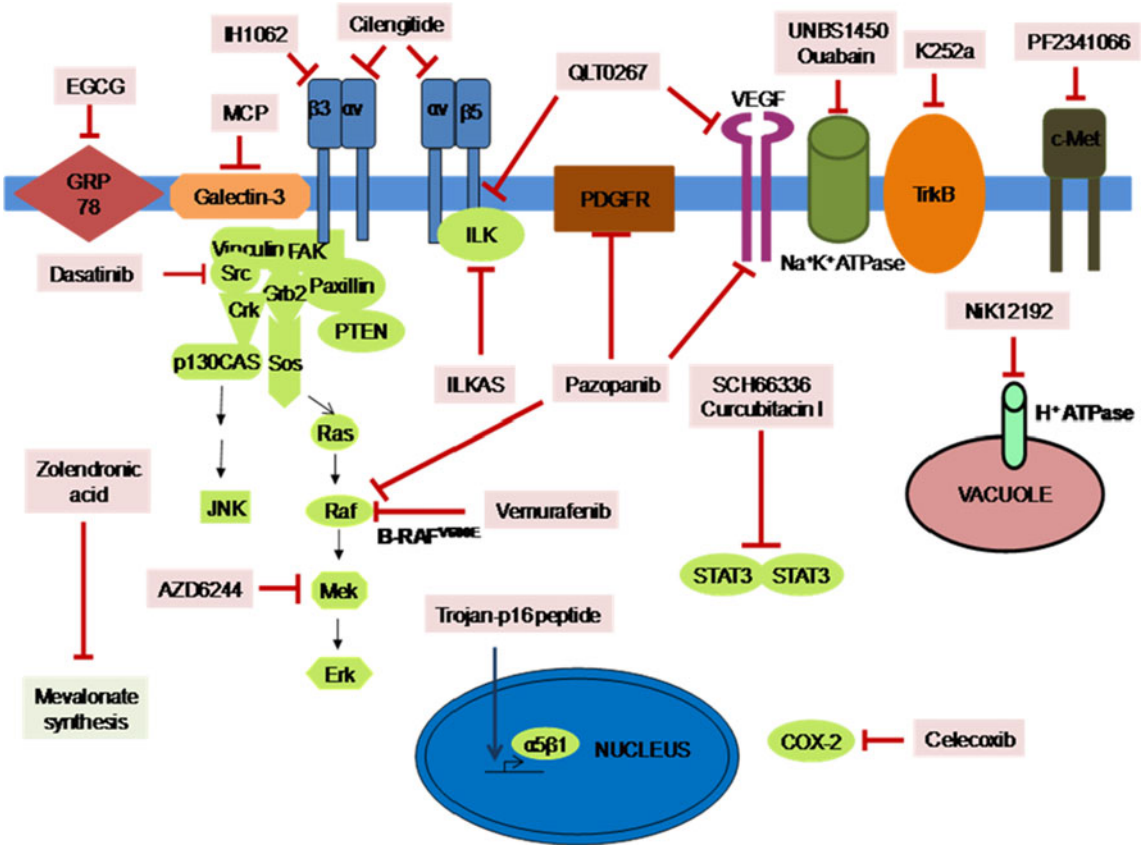


Fig. 1 Novel anti-cancer compounds targeting anoikis regulating receptors and intracellular signal transducers. The mechanisms of action of various drugs and anti-cancer lead compounds represent potential opportunities for devising individualized and targeted combinatorial regimens.

Targeting αvβ3/αvβ5/α5β1 Integrins and Integrin-Linked Kinase (ILK) Signaling in Glioblastoma Multiforme (GBM)

Glioblastoma multiforme (GBM) is an aggressive primary brain tumor with a very poor median survival of 16.2 months with chemo-radiotherapy (32). Temozolomide (TMZ) is the contemporary drug of choice that is extensively used in the clinical management of GBM, but resistance to TMZ is very much prevalent. The expression of αvβ3 and αvβ5 integrins is associated with aggressive histological grade and neo-angiogenesis in GBM (33). In this regard, inhibition of αvβ3 and αvβ5 integrins has received significant attention in developing potential interventions for GBM. Cilengitide (EMD 121974), a cyclized pentapeptide [Arg-Gly-Asp-DPhe-(NMeVal)], can inhibit both αvβ3 and αvβ5 integrins in GBM (34). Interestingly, cilengitide co-treatment enhanced the anti-cancer effects of TMZ while on chemo-radiotherapy consisting of TMZ and external beam radiation therapy (XRT) (34). An advantageous feature of cilengitide is the specific inhibition of αvβ3 and αvβ5 integrins. The integrin inhibition following cilengitide treatment was well tolerated and did not affect the associated processes like platelet aggregation, which is regulated by normal glycoprotein IIb/IIIa-mediated attachment to fibrinogen (35).

The tumorigenic GBM spheres also have high α5β1integrin expression (36). A novel α5β1 integrin inhibitor called JSM6427 has shown potential anti-tumor effects in *in vivo* models (37). Activation of integrin-linked kinase (ILK) leads to anoikis resistance and facilitates metastases (38). Some of the ILK-targeted studies have also been pursued to inhibit migration and invasion of GBM (39,40). ILKAS (ISIS Pharmaceuticals Inc.), an antisense-based formulation that can target ILK in GBM, has shown potent anti-tumor effects in *in vivo* studies (39). Another small molecule ILK inhibitor, QLT0267, has also been very effective in inducing GBM tumor regression by inhibiting ILK and VEGF expression (40). These advances have expanded the choices for development of TMZ and integrin-targeted combinatorial therapies to target drug-resistance in GBM.

Role of Galectin-3/Glucose Regulated Protein 78 (GRP78)-Targeted Interventions in Anoikis-Resistant Breast Cancer

Caucasian women are more susceptible to breast cancer than Asian women (41). A recent epidemiological study revealed that a functional germ line mutation in galectin-3 gene at position 191 leads to substitution of proline with histidine at the 64th amino acid of galectin-3 protein. The proline substitution in galectin-3 showed a positive association with Caucasians, increased incidence of breast cancer and acquisition of drug resistance (42). Galectin-3 belongs to the family of β-galactoside binding lectins, which are involved in the regulation of proliferation, apoptosis and tumor growth (43–45). A significant feature of the galectin-3 gene is the presence of four amino acids motif (Asp-Trp-Gly-Arg; NWGR) that is also conserved in the BH1 domain of the anti-apoptotic bcl-2 gene family, which functions in inducing the anti-apoptotic effects of galectin-3 (46). Hence, galectin-3 is a novel target for breast cancer interventions in Caucasian population. Galectin-3 overexpression is also associated with anoikis resistance in human breast cancer cells (47). A galectin-3 inhibitor, modified citrus pectin (MCP) that is enriched in β-galactosidase is being investigated in breast cancer. The administration of MCP leads to decreased formation of metastatic colonies of breast and prostate cancer cells in mice tail-vein anoikis model (48).

The expression of glucose regulated protein 78 (GRP78) positively predicts resistance to taxols and negatively predicts the "time to recurrence" (TTR) in 67% of breast cancers (49). The GRP78 protein is a critical regulator of endoplasmic reticulum (ER) functions like protein folding, initiating unfolded protein response and controlling the initiation of ER stress response (50,51). The tumor microenvironment has a state similar to physiological conditions that induce ER stress, and in this regard the up regulation of GRP78 is critical for tumor survival (52,53). The expression of GRP78 is associated with resistance to many chemotherapeutic drugs like adriamycin, etoposide and paclitaxel in breast cancers (49,54,55). Recently, it was also shown that GRP78 leads to anoikis resistance by interacting with Cripto, a small, glycosyl-phosphatidylinositol-anchored signaling protein involved in EMT and migration (56). A green tea constituent called epigallocatechin-3 galate (EGCG) can effectively inhibit GRP78 expression and sensitize the breast cancer cells to chemotherapy agents like paclitaxel and vinblastine (57). Thus, galectin-3 and GRP78 represent significant targets to develop combinatorial therapies for drug and anoikis resistance of breast cancer in Caucasian patients.

Role of α5β1 Integrin/p16^{INK4a} Signaling Axis in Targeting Anoikis Resistance in Lymphoma and Pancreatic Cancers

The β1 integrin subunit is known to induce resistance to DNA-damaging drugs like mitoxantrone and etoposide in human lymphoma cells (58). The β1 integrin-induced resistance to mitoxantrone and etoposide was associated with increased binding of the DNA repair protein topoisomerase II to the DNA, which in turn would explain the decreased susceptibility of targeted tumors to respective drugs (58). Curcumin, an active anti-cancer compound being tested in phase I–III trials in myeloma, lung, pancreatic and colon

cancers, is a known anoikis-inducing agent, which predominantly acts by inhibiting the anti-apoptotic protein Bcl2 (59). Curcumin is also known to effectively inhibit the expression of topoisomerase-II (60). Tetrahydro curcumin (THC), a major metabolite of curcumin, can effectively target the mitoxantrone resistance protein (MXR or ABCG2)-expressing cells and sensitize them to mitoxantrone (61). Thus, the multi-targeting potential of curcumin and its analogues make them novel candidates for developing combinatorial therapies in anoikis- and mitoxantrone-resistant tumors with high β1 integrin subunit expression.

The expression of α5β1 integrin shows a positive correlation with tumor progression in some cancers like GBM, whereas loss of α5β1 integrin is observed in some tumors like pancreatic cancer (36,62). The aberrant expression of α5β1 integrin is often mediated by inherent genetic abnormalities in the $p16^{INK4a}$ tumor suppressor. The loss of $p16^{INK4a}$ along with gain in telomerase activity is an early event that promotes the oncogenic transformation of normal cells (63). The $p16^{INK4a}$ is frequently lost in pancreatic adenocarcinomas along with the loss of α5β1 integrin expression (62). Transfection of $p16^{INK4a}$ restores anoikis sensitivity along with inducing the re-expression of α5β1 integrin in pancreatic adenocarcinoma cells (44,64). In one of the studies, a carrier sequence consisting of antennapedia homeodomain (Cys-RQIKIWFQNRRMKWKK, Trojan peptide) was conjugated to a 21-residue synthetic peptide corresponding to 84–103 amino acids of $p16^{INK4a}$ along with an additional cysteine (DAAREGFLDTLVVLHRAGAR-Cys, p16 peptide) to synthesis the Trojan-p16 peptide. The Trojan-p16 peptide was highly effective in reaching both cytoplasmic and nuclear compartments of target cells and exerted potent anti-tumor effects in *in-vivo* studies. Thus, therapeutic peptides like Trojan-p16 represent a relevant strategy for delivering $p16^{INK4a}$ to restore anoikis sensitivity in pancreatic tumors with loss of $p16^{INK4a}$ tumor suppressor (44,64).

ROLE OF DRUGS TARGETING TYROSINE KINASE NETWORKS IN ANOIKIS RESISTANCE

Targeting TrkB/STAT3/EGFR/c-Src/HGF/COX-2 Signaling in Anoikis-Resistant Head and Neck Cancers

Overexpression of tyrosine kinase receptors and activation of downstream signaling cascades are involved in regulation of anoikis resistance (65). A genome-wide retroviral cDNA screening to identify anoikis-inducing genes in anoikis-sensitive rat intestinal epithelial (RIE) cells identified a neurotrophic tyrosine kinase receptor, tropomyosin related kinase-B (TrkB), as a potential molecular antagonist of anoikis (66). TrkB induces EMT and enhances anoikis resistance by up-regulating the activation of PI3K, AKT and by increasing the expression of transcription factors twist and snail, which are associated with anoikis-resistance (18). TrkB facilitates the formation of large cellular aggregates that survive and proliferate in suspension. The tumors formed following inoculation of TrkB-transfected immortalized kidney epithelial cells in mice revealed increased tumorigenicity and metastatic spread along with a decrease in the number of apoptotic cells in tumors colonized to distant organs, in comparison to tumors where TrkB down-stream signaling was inactivated by treatment with shRNA for twist, a mediator of TrkB-induced EMT (18). TrkB expression is also known to correlate with aggressive metastatic behavior and poor clinical prognosis (67).

BDNF/TrkB signaling is frequently activated in Epstein-Barr virus (EBV)-induced nasopharyngeal carcinoma cells, which are known for their characteristic anoikis resistance and metastatic phenotype (68). Treatment with K252a, an indalocarbazole TrkB inhibitor, sensitized the nasopharyngeal carcinoma cells to anoikis (68). Further investigations on pan Trk (Trk A, B and C) inhibitors like AZ-23 (AstraZeneca, Boston, MA) and newly developed small peptide TrkB inhibitor cyclotraxin-B, which can inhibit both BDNF-dependent and -independent activation of TrkB, would provide more therapeutic options for targeting BDNF/TrkB pathway in nasopharyngeal carcinoma (68–70). BDNF/TrkB signaling is also known to activate STAT3 (71). The STAT3 signaling is constitutively active in nasopharyngeal carcinomas and head and neck squamous cell carcinomas (72,73). Activation of STAT3 is known to cause increased migration and anoikis resistance induced by endothelial secretory factors like interleukin-6 (IL-6) and Chemokine (C-X-C motif) ligand-8 (CXCL-8) (74). Curcubitacin I, a natural and selective inhibitor of JAK/STAT3 pathway, induces anoikis and inhibits invasive potential of nasopharyngeal carcinoma cells. Hence, targeting TrkB and JAK/STAT3 signaling would be a mechanistically sound strategy to reactivate anoikis sensitivity and inhibit metastatic progression in nasopharyngeal carcinomas (75).

Head and neck squamous cell carcinomas (HNSCC) are one of the highly anoikis-resistant metastatic tumors (76). HNSCC express high levels of epidermal growth factor receptor (EGFR), and EGFR inhibitors were initially used to target HNSCC (77). But, the resistance to EGFR inhibitors is very high due to EGFR mutations, progressive EMT-induced changes and dependence of HNSCC on other signaling cascades (78,79). Hepatocyte growth factor (HGF) and its receptor c-Met are over-expressed in HNSCC and play a vital role in the anoikis resistance of HNSCC. HGF/c-Met signaling induces anoikis resistance in HNSCC via the activation of AKT and ERK, independent of NF-κB-mediated transcriptional re-programing (80). HNSCC also shows increased expression of the oncogene c-

Src, which is known to transduce receptor tyrosine kinase signals (81). The oncogene c-Src is known to be an upstream regulator of c-Met, and the c-Src inhibitor, dasatinib, also causes c-Met inhibition along with targeting c-Src-sensitive cells. Interestingly, in a subset of HNSCC cells that are non-responsive to anti-proliferative effects of c-Src inhibitors, c-Src was actually inhibited but the down-stream c-Met inhibition was absent (82). In one of the studies on HNSCC, treatment with EGFR inhibitor, erlotinib, also lead to partial c-Met inactivation, and the combination of erlotinib and dasatinib was better than single agent treatment (82). A recent phase II trial on dasatinib alone did not reveal substantial benefit in HNSCC (83). In another study on HNSCC, the combination of c-Met inhibitor, PF2341066, and EGFR inhibitor, gefitinib, induced potent anti-cancer effects compared to either of the single agents (84). Thus, c-Met inhibition is a significant factor for targeting both proliferation and anoikis resistance, than c-Src or EGFR alone, in HNSCC.

The activation of HGF/c-Met axis also enhances the expression of cyclooxygenase-2 (COX-2), which is involved in regulating anoikis in HNSCC (85). The expression of COX-2 leads to increased expression of VEGF and prostaglandin E2 (PGE2), which in turn activates the expression of snail (86). Snail is a transcription factor that can reduce the expression of E-cadherin, an epithelial marker and potential tumor suppressor, and induce EMT in epithelial cancers (18). As COX-2 is an HGF/C-Met-induced anoikis effector, targeting COX-2 would be a rational choice for developing combinatorial therapies given the significant dependency of HNSCC on HGF/c-Met signaling. A recent phase I clinical trial on combining COX-2 inhibitor, celecoxib, and EGFR inhibitor, erlotinib, with irradiation has revealed that the combination is well tolerated at therapeutically effective doses in recurrent HNSCC (87). Additional anoikis-targeted interventions in HNSCC include studies on copper chelators. Treatment with tetrathiomolybdate, a copper chelator, has shown to effectively target the survival of HNSCC and restore anoikis sensitivity in pre-clinical studies (88). In this regard, further integrated approaches for targeting EGFR/c-Src/c-Met/COX-2 signaling cascades represent rational opportunities for developing effective interventions for HNSCC.

Targeting Dynamic Changes in EGFR/PDGFR Signaling in Anoikis-Resistant Non-small Cell Lung Cancer (NSCLC)

EGFR mutations are common in many tumors of lung and are predictive of response to specific EGFR inhibitors (89). The small molecule EGFR inhibitors, erlotinib and gefitinib, are more effective in lung cancer patients with EGFR mutations, compared to EGFR antibody, cetuximab, which is more effective in lung cancers with wild-type EGFR (89,90). The loss of tumor suppressor PTEN is associated with aggressive and anoikis-resistant progression of lung cancer (91). Recent studies have revealed that a synthetic derivative of curcumin called difluorinated-curcumin (CDF) can enhance the PTEN expression along with inhibiting NF-κB (92). Interestingly, in lung cancer patients with gefitinib resistance, PPAR-γ agonist rosiglitazone induced the expression of PTEN and enhanced sensitivity to gefitinib (93).

In non-small cell lung cancer (NSCLC), receptor tyrosine kinase signaling undergoes dynamic changes for favoring survival of tumors during transition from epithelial (epithelial NSCLC) to mesenchymal (mesenchymal NSCLC) phenotype with progressive induction of EMT, an established molecular event during acquisition of anoikis resistance (4,10,18,94). The mesenchymal NSCLC is less dependent on EGFR signaling as revealed by reduction in basal phosphorylation of EGFR along with a decrease in EGF production (94). Usually, epithelial NSCLC do not express platelet derived growth factor receptors (PDGFR) whereas mesenchymal NSCLC acquire PDGFR expression (94). Also, the activation of downstream kinases like PI3K, SHP2 and STAT3 are enhanced in mesenchymal NSCLC compared to epithelial NSCLC (94). Treatment with EGFR inhibitor, erlotinib, in mesenchymal NSCLC with low EGFR activity, leads to enhanced phosphorylation of PDGFR, which indicates that PDGFR activation is a compensatory response to inhibition of residual EGFR activity in mesenchymal NSCLC (94). The up-regulation of PDGFR in mesenchymal NSCLC is significant as PDGFR specifically regulates the activation of Src kinase in suspended conditions and confers anoikis resistance in lung cancers (95).

The changes in the dependence of NSCLC from EGFR to PDGFR/Src signaling with induction of EMT represent potential opportunities for targeting the anoikis resistance of NSCLC. Hence, PDGFR inhibitors and drugs inhibiting PI3K, STAT3 and Src would be of potential significance in anoikis-resistant NSCLC. In this regard, pazopanib (Votrient; GlaxoSmithKline), an orally active multi-targeted inhibitor of PDGFR-α/β, VEGFR-1/2/3, and c-kit, which was approved by FDA in 2009, has demonstrated promising anti-cancer activity in a phase II clinical trial on NSCLC (96). Tyrosine kinases activate PI3K/mammalian target of rapamycin (mTOR) pathway, which regulates the transduction of signals from growth factor receptors. Though targeting mTOR pathway is desirable in PDGFR-dependent and anoikis-resistant NSCLC, due to feed-back activation of AKT/MAPK pathway upon mTOR inhibition and presence of frequent activating K-RAS mutations in NSCLC, additional interventions downstream of K-RAS like the use of MEK/ERK inhibitors become essential as part of developing combinatorial therapies for NSCLC (97). Recent development of NVP-BEZ235, a dual pan-PI3K and downstream mTOR inhibitor, is another promising drug for targeting NSCLC. A Pre-clinical trial on the combination of NVP-BEZ235 and the MEK inhibitor,

AZD6244/ARRY-142886, has shown that the combination can target even K-RAS-mutant lung cancers (98). As STAT3 activation can potentiate resistance to MEK inhibition, testing STAT3 inhibitors along with various combinations to target late-stage mesenchymal NSCLC, which express high levels of PDGFR, PI3K and STAT3, would be a relevant combinatorial strategy to ensure better therapeutic response (99). SCH66336 is a small molecule inhibitor of STAT3, which has shown anti-cancer activity in lung cancers (100). Dr. Jing's research group has developed a novel class of G-quarted oligodeoxynucleotide (GQ-ODN) STAT3 inhibitors, T40214 and T40231, which are effective in causing regression of established NSCLC tumors (101). Further rational combinations of EGFR/PDGFR/PI3K/AKT/mTOR/STAT3 signaling inhibitors and chemotherapy drugs, depending on epithelial or mesenchymal histology of the diagnosed lung tumors, would help to target anoikis-resistant metastatic progression of NSCLC.

ROLE OF DRUGS MODULATING OXIDATIVE-STRESS, OSMOTIC-STRESS AND METABOLIC PATHWAYS IN ANOIKIS RESISTANCE

Oxidative-stress regulates a plethora of cellular events including anoikis. A cross-talk between cell-matrix sensors and cellular anti-oxidant networks to buffer oxidative-stress was systematically demonstrated in a comprehensive study by Brozovic A *et al.* (102). In this study, αvβ3 integrin-negative human laryngeal carcinoma (HEp2) cells were transfected with αvβ3, an integrin that mediates anoikis resistance, and glutathione (GSH) and reactive oxygen species (ROS) levels were analyzed. The transfection of αvβ3 integrin lead to a strong increase in the levels of GSH and a decrease in the levels of ROS in HEp2-αvβ3 cells compared to control HEp2 cells (102). Thus, integrins favoring anoikis resistance appear to have a distinct role in regulating the cellular anti-oxidant networks. Integrin-induced signals transduce through adaptor proteins like ILK-FAK-Grb2-SOS complex to activate Ras, which in turn activates down-stream RAF, Ral and PI3K pathways (4–6). Studies in breast and colon cancer have revealed that Ral activation leads to anchorage-independent growth of cancer cells (103).

Role of Ral-Binding Protein 1 (RalBP1 or RLIP76)-Targeted Interventions in Anoikis-Resistant Metastatic Tumors

Extensive studies have characterized the tumor promoting and metastasis favoring nature of Ral-binding protein 1 (RalBP1 or RLIP76), a 76 kDa multi-specific Ral-effector that also predominantly functions as a transporter of glutathione-conjugates (GS-E) of chemotherapy drugs and toxic end products of lipid peroxidation (104,105). In our extensive studies, depletion of RLIP76 by R508 phosphorothioate antisense or inhibition of the transport activity by RLIP76 antibody effectively inhibited the proliferative and migratory capacity of melanomas, neuroblastomas, squamous cell carcinomas and tumors of lung, kidney and prostate. RLIP76 inhibition also substantially sensitized the tumor cells to radiation and chemotherapy drugs like doxorubicin, cisplatin, sorafenib and sunitinib (105,106). Targeting RLIP76 has been very effective in inhibiting anoikis-resistant metastatic progression of bladder and prostate cancers (107). Some of the anti-diabetic drugs like metformin and rosiglitazone inhibit the transport of GSH-conjugates of lipid peroxidation products like 4-hydroxynonenal (4HNE) by RLIP76 (108). According to a prospective study, metformin used for the management of diabetes mellitus and metabolic syndrome also reduces the incidence of cancer risk (109). Further studies can reveal whether the combination of metformin and RLIP76-directed combinatorial interventions would be a promising strategy to target the incidence and progression of anoikis-resistant cancers in patients with co-morbid diseases like diabetes mellitus.

Role of Targeting Superoxide Anion Production and Associated Epigenetic Methylation in Anoikis Resistance

Recent studies have implicated oxidative-stress and superoxide anions in epigenetic events that regulate anchorage-independent progression in cancers. Melanoma cell lines established by serial subcultures of anchorage blockade have increased superoxide anion production, high levels of expression of DNA methyl transferases (DNMTs), DNMT1 and DNMT3b, and DNA methylation (110). This finding, for the first time, revealed the potential role of DNMTs in anoikis resistance of tumors. The treatment of melanoma cells with N-acetyl cysteine (NAC) and l^G-Nitro-l-arginine methyl ester (L-NAME) further lead to anoikis sensitivity that was associated with a decrease in DNA methylation and inhibition of superoxide anion production during anchorage independent growth (110). In a subpopulation of hormone refractory prostate cancers (HRPC), there is increased DNMT1 and DNMT3b expression along with the presence of enhanced resistance to docetaxel and cisplatin. The treatment with azacytidine leads to consistent decrease in DNMT1 and DNMT3b expression along with sensitizing the HRPC cells to docetaxel and cisplatin (111). Epigallocatechin-3 galate (EGCG), a green tea constituent, which can directly bind to DNMTs and inhibit the enzymatic activity, is being investigated as potential dietary supplement in many cancer patients (112). Hence, the rational combination of specific anti-oxidants like NAC, L-

NAME, EGCG and chemotherapy drugs can lead to personalized and targeted therapies in patients with epigenetic methylations and drug resistance.

Potential Role of Na^+/K^+ ATPase and Vacuolar -H^+ ATPases-Directed Interventions in Targeting Anoikis Resistance

The survival of anoikis-resistant cells in suspended conditions, while traversing through blood vessels, requires modulation of signaling proteins regulating osmotic-stress. A normal Na^+ and osmotic gradient is maintained by Na^+/K^+ ATPase pump, which has increased expression and activity in many cancers (113). The β subunit of Na^+/K^+ ATPase pump is often down-regulated while α subunits are up-regulated in cancers (114,115). In this context, Na^+/K^+ ATPase pump inhibitors like cardiac glycosides, due to their predominant binding to α subunits of the Na^+/K^+ ATPase pump, become significant for anti-cancer interventions (116). In one of the studies, screening of around 2000 compounds lead to identification of ouabain, digoxin, digitoxin, peruvoside and strophanthidin as potential anoikis-sensitizing agents in anoikis-resistant PPC-1 prostate cancer cells. Ouabain effectively inhibited anoikis resistance *in vitro* and decreased distant tumor colonization in *in vivo* models of prostate cancer. Overexpression of Na^+/K^+ ATPase pump reversed the ouabain-induced reduction in metastatic potential (117). Some of the recent analogues of cardiac glycosides do target other anti-cancer pathways, which would facilitate tolerable dosage formulations with appropriate combination with other anti-cancer agents. Activation of NF-κB leads to anoikis resistance in suspended conditions (118). UNBS1450, a hemi-synthetic derivative of the novel cardenolide called 2″-oxovoruscharin, can target NF-κB along with inhibiting Na^+/K^+ ATPase pump in lung cancer. UNBS1450 was more effective than cisplatin, carboplatin and oxaliplatin in lung cancer models, and is being investigated in phase I clinical trials for solid tumors (119)

Vacuolar-H^+ ATPases play a critical role in the maintenance of cellular proton balance and intracellular redox potential, which in turn, regulates invasive and metastatic behavior of cells. The 16 kDa subunit C of vacuolar H^+ ATPase was previously shown to interact with β1 integrin subunits, which are generally associated with adhesion-dependent signal transduction (120). NiK-12192, a vacuolar-H^+ ATPase inhibitor, induced growth inhibition similar to anoikis induction in colon cancer cells (121). Further studies on Na^+/K^+ ATPase and vacuolar-H^+ ATPase inhibitors as single agents and in combination with other anti-cancer drugs would determine the significance of osmotic-stress-targeted interventions for the management of anoikis-resistant tumors.

Potential Role of Mevalonate Pathway-Targeted Interventions in Anoikis-Resistant Osteosarcomas

Mevalonate pathway that is involved in cellular generation of cholesterol and isoprenoid lipids like geranyl and farnesyl pyrophosphates has significant therapeutic implications (122). Zolendronic acid (ZOL) is a third generation nitrogen-containing biphosphonate and an inhibitor of the mevalonate pathway (123). In osteosarcoma, ZOL induces anoikis sensitivity that can be reversed by the supplementation with geranylgeraniol, which signifies the role of mevalonate pathway in regulating anoikis resistance in these tumors. ZOL appears to be a potential anoikis-targeting agent for osteosarcomas with favorable pharmacokinetics, as the administration of even a high dose of 4 mg lead to only 2 μM serum concentrations, whereas maximum fraction of administered dose reaches the bone (124). The overexpression of p-glycoprotein, which is associated with multidrug resistance in osteosarcomas, did not affect the sensitivity of osteosarcomas to ZOL (125). Thus, mevalonate pathway inhibitors like ZOL have significant potential to target anoikis-resistant osteosarcomas with high p-glycoprotein expression.

COMBINATORIAL DRUG DEVELOPMENT STRATEGIES IN CANCER THERAPY

The ability of anoikis-resistant cancer cells to continuously adapt to their microenvironment and initiate changes in cell signaling cascades leads to acquisition of high metastatic potential in tumors (2,3,18,19). The inherent molecular complexity, number of current clinical cancer cases and an estimated 55% rise in the incidence of cancers by the year 2025, together necessitate more effective, integrated and targeted therapies (126). Currently, the majority of combinatorial studies are driven by the clinical need for more effective therapies due to lack of uniform response, emergence of resistance and dose-limiting toxicities (127). The current drug development and anti-cancer therapies are in an accelerated phase of transition from single-target interventions, and are gaining momentum towards multi-target drugs and combinatorial therapeutics (128).

The combinatorial cancer therapeutics requires extensive resources and multi-disciplinary teams to ensure optimum and tumor-selective targeting of the differentially-regulated signaling networks. But, when compared to single target interventions, such approaches are mechanistically stronger and represent more effective research strategies for optimizing the resources of drug development from initial pre-clinical stages to clinical drug development. Such effective strategies result from integrated and multi-parametric assessment of tumor tissue type, grade of

Table I Novel Anti-cancer Compounds Targeting Anoikis-Resistant Tumors

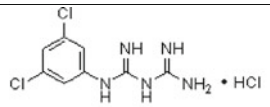
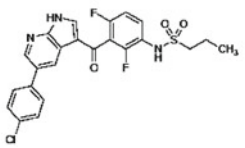
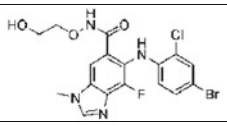
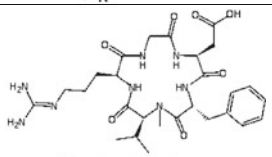
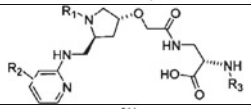
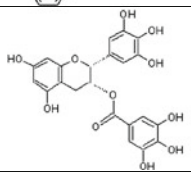
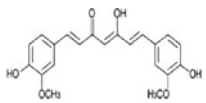
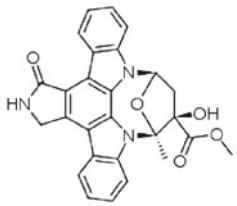
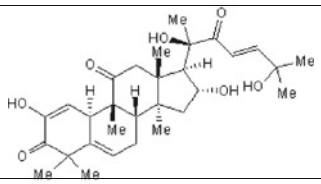
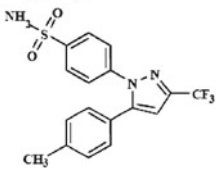
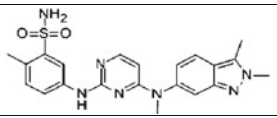
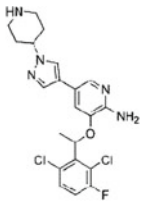
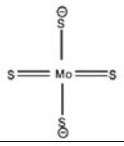
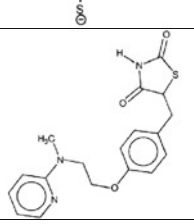
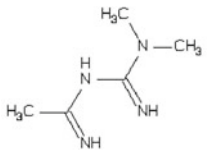
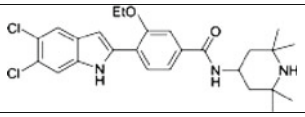
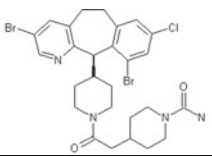
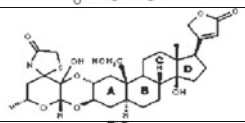
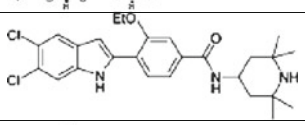
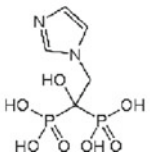
Name of anti -cancer compound	Structure of the compound	Type of cancer	Major effects on anoikis signaling
IH1062 (3, 5-dichloro-phenylbiguanide)		Melanoma	Inhibitor of αvβ3 integrin leading to decreased FAK activation (28)
Vemurafenib		Melanoma	B-RAF ^{V600E} inhibitor (29)
AZD6244		Melanoma	MEK inhibitor. Benefit in B-RAF ^{V600E} expressing melanoma (30, 31)
Cilengitide [Ar g-Gly -Asp-DPhe-(NMeVal)]		Glioblastoma multiforme	Inhibitor of αvβ3 and αvβ5 integrins, effective alone and in combination with TMZ (34)
JSM6427		Glioblastoma multiforme	Inhibitor of α5vβ1 integrin (37)
Epigallocatechin -3 galate (EGCG)		Breast cancer, Prostate cancer	Inhibitor of GRP78 and DNMT. Sensitizes to paclitaxel and vinblastin (57)
Curcumin		Lymphoma, Myeloma, pancreatic cancer, colon cancer, Non small cell lung cancer	Inhibitor of Bcl2, DNA topoisomerase II Tetrahydrocurcumin inhibits mitoxantrone resistant protein [59-61], Diflurinated curcumin increases PTEN (92)
K252a		Nasopharyngeal carcinoma	Inhibits TrkB (68)
Curcubitacin I		Nasopharyngeal carcinoma	Inhibitor of STAT3 (71)
Celecoxib		Head and neck squamous cell carcinoma	Inhibitor of COX-2 (87)
Pazopanib		Non-small cell lung cancer, human breast cancer	Inhibitor of VEGFR -1/2/3, PDGFR-α/β, c-kit. (96)

Table I (continued)

Name of anti -cancer compound	Structure of compound	Type of cancer	Major effects on anoikis signaling
PF2341066		Head and neck squamous cell carcinoma	Inhibitor of c-Met (84)
Tetrathiomolybdate		Head and neck squamous cell carcinoma	Copper chelator (88)
Rosiglitazone		Non-small cell lung cancer	Anti-diabetic drug which increases PTEN (93), inhibits RLIP76 transport function (108)
Metformin		Bladder cancer	Decreases risk of bladder cancer, inhibits RLIP76 transport function (108,109)
NiK-12192		Colon cancer	Inhibition of vacuolar -H+ ATPase (121)
SCH66336		Non-small cell lung cancer	Inhibitor of STAT3 (100)
UNBS1450		Prostate cancer	Inhibition of Na^+/K^+ ATPase pump (119)
NiK-12192		Colon cancer	Inhibition of vacuolar -H^+ ATPase (121)
Zolendronic acid		Osteosarcoma	Inhibits farnesyl diphosphate synthase in mevalonate pathway (124,125)

disease, toxicities associated with conventional clinical drugs and presence of co-morbid diseases. Also, the preferential signaling dependency of a tumor at a specific stage and proximate associated signaling cascades, which can activated consequent to targeting initial tumor preferences in survival signaling, needs to be considered. In this context, the various anoikis resistance-targeting compounds represent novel opportunities for pursuing such integrated and combinatorial drug development (Table I).

Significance of Collective Targeted Approaches in Advanced and Anoikis Resistant Cancers

In melanomas, the tumor growth phases include "radial growth phase (RGP)" and "vertical growth phase (VGP)" (129). Melanomas behave as highly metastatic tumors after invading the dermis and hence, malignant melanomas in RGP are usually surgically excised but post-surgical recurrence is common from satellite lesions, which become aggressive once the primary tumor is removed (129). Many of the molecular events like increased expression of αvβ3 integrin and B-RAF associated with VGP initiate anoikis-resistance as a survival mechanism for melanomas in non-native dermal matrix (25,26,130). Thus, pre-operative and post-operative anoikis-targeted interventions for inhibiting αvβ3 integrin/B-RAF/MEK expression represent rational choices to achieve effective tumor clearance and prevent recurrence in malignant melanomas. Glioblastoma multiforme (GBM) is another tumor which has characteristically diffuse borders and high metastatic potential which together present challenges for effective tumor resection (131). In this regard, the collective targeted strategies for integrin and ILK signaling in GBM would be a novel choice to enhance the surgical outcomes and prevent recurrence in GBM.

Dynamic calibration of signaling cascades with changes in morphological phenotype and metastatic progression of tumors is another area of interventional focus in cancers. For example, the anti-cancer drugs like erlotinib or gefitinib

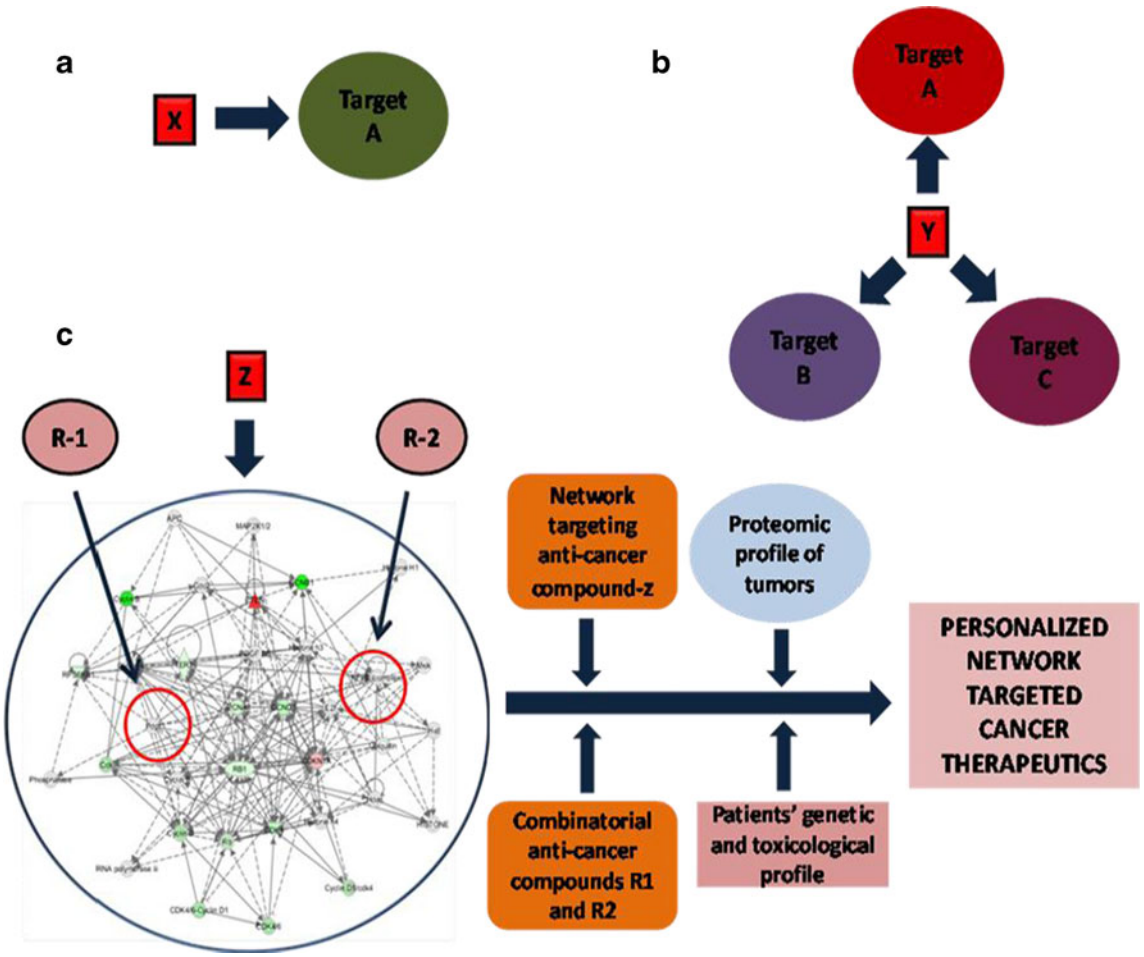


Fig. 2 Schematic diagram representing the significance of personalized network targeted cancer therapeutics The anti-cancer drugs like erlotinib or gefitinib were used in the treatment of EGFR expressing tumors including NSCLC (**a**, "X"). The next generation of drugs like pazopanib, an orally active multi-targeted inhibitor of PDGFR-α/β, VEGFR-1/2/3, and c-kit represent an advanced class of drugs that can be considered to target switch from EGFR to PDGFR as well as increased VEGFR expression in NSCLC (**b**, "Y"). Further trials on new class of anti-cancer agents like NVP-BEZ235, a new orally active dual pan-PI3K and downstream mTOR inhibitor ("Z") along with a potential combination with other compounds like inhibitors of STAT3 ("R-1") and inhibitors of Src ("R-2") would be relevant strategies to target the distinct tumor signaling profile in mesenchymal NSCLC having additional activation of STAT3 and Src. The choice of specific anti-cancer compounds can be further optimized in individual patients depending on the patients' genetic and toxicological profile as well as proteomic and genomic characterization of incident tumors for elucidating the differential protein expression and presence of mutations, respectively (**c**).

were used in the treatment of EGFR-expressing tumors including NSCLC (89,90). EMT associated with anoikis resistance leads to switch from EGFR to increased PDGFR expression and mesenchymal phenotype in advanced NSCLC (71). The next generation of drugs like pazopanib, an orally active multi-targeted inhibitor of PDGFR-α/β, VEGFR-1/2/3 and c-kit represent an advanced class of drugs that can be considered to target the switch from EGFR to PDGFR as well as increased VEGFR expression in NSCLC (96). But, the switch from EGFR to PDGFR is also associated with increase in downstream STAT3 and Src signaling in mesenchymal NSCLC (94). Hence, further trials on new class of anti-cancer agents like NVP-BEZ235, a new orally active dual pan–PI3K and downstream mTOR inhibitor along with a potential combination with other compounds like inhibitors of STAT3 and Src would be relevant strategies to target mesenchymal NSCLC (98–101). The choice of specific anti-cancer compounds can be further optimized in individual patients depending on the patients' genetic and toxicological profile as well as proteomic and genomic characterization of incident tumors. Such integration of distinct tumor signaling and patient profiles will lead to effective personalized network targeted cancer therapeutics (Fig. 2).

Resources for Future Development of Effective Combinatorial Cancer Therapies

Development of proteomics utilizing ultra-high sensitive mass spectrometry methods and wider application of proteomic softwares like Ingenuity pathway analyses (IPA, Ingenuity Systems) have amplified the technical potential to elucidate the distinct regulation of complex networks in specific genotypes of cancer along with revolutionizing the identification of differentially-regulated signaling networks that are predictive of therapeutic responses (132). Based on such integration of signaling network parameters, we recently characterized and reported the anti-cancer effects of a novel and highly effective flavonoid vicinen-2 as a single agent and in synergistic combination with docetaxel in prostate cancer (133). The results from NCI-60 panel, based on testing large number of chemicals as part of studies under developmental therapeutics program, have expanded the knowledge database and contributed to the formulation of COMPARE algorithm for analyzing potential response to intended anti-cancer agents before pursuing elaborate testing of respective agents (134). The TheraScreen EGFR 29 Mutation Test, KRAS LightMix (TIB MolBiol) and TheraScreen K-RAS Mutation Kits (DxS Ltd.) are some of the commercially available tests that help to evaluate EGFR and K-RAS mutation status in patients with solid tumors (135,136).

In view of advances in the molecular understanding of dynamic changes in tumor signaling profiles and parallel development of screening tests to predict the choice of specific anti-cancer agents, personalized and network-targeted therapies are becoming increasingly essential. Such pursuit of innovative research strategies by rationally incorporating combinatorial drugs, in the context of specific signaling network adaptations and dependencies in respective tumors, would enhance the productivity and translational relevance of drug development. Further trials on the specific combinations of anti-cancer compounds in patients with distinct genotypes and drug-sensitivity profiles will facilitate the development of interventional strategies based on advanced combinatorial cancer therapeutics.

ACKNOWLEDGMENTS & DISCLOSURES

This work was supported in part by USPHS grant CA 77495 and Cancer Research Foundation of North Texas.

REFERENCES

1. Frisch SM, Ruoslahti E. Integrins and anoikis. Curr Opin Cell Biol. 1997;9:701–6.
2. Nagaprashantha LD, Vatsyayan R, Lelsani PC, Awasthi S, Singhal SS. The sensors and regulators of cell-matrix surveillance in anoikis-resistance of tumors. Int J Cancer. 2011;128:743–52.
3. Frisch SM, Screaton RA. Anoikis mechanisms. Curr Opin Cell Biol. 2001;13:555–62.
4. Loftus JC, Liddington RC. Cell adhesion in vascular biology. New insights into integrin–ligand interaction. J Clin Invest. 1997;99:2302–6.
5. Calalb MB, Polte TR, Hanks SK. Tyrosine phosphorylation of focal adhesion kinase at sites in the catalytic domain regulates kinase activity: a role for Src family kinases. Mol Cell Biol. 1995;15:954–63.
6. Cohen LA, Guan JL. Mechanisms of focal adhesion kinase regulation. Curr Cancer Drug Targets. 2005;5:629–43.
7. Ley R, Balmanno K, Hadfield K, Weston C, Cook SJ. Activation of the ERK1/2 signaling pathway promotes phosphorylation and proteasome-dependent degradation of the BH3-only protein, Bim. J Biol Chem. 2003;278:18811–6.
8. Almeida EA, Ilic D, Han Q, Hauck CR, Jin F, Kawakatsu H, *et al.* Matrix survival signaling: from fibronectin via focal adhesion kinase to c-Jun NH(2)-terminal kinase. J Cell Biol. 2000;149:741–54.
9. Duxbury MS, Ito H, Zinner MJ, Ashley SW, Whang EE. Focal adhesion kinase gene silencing promotes anoikis and suppresses metastasis of human pancreatic adenocarcinoma cells. Surgery. 2004;135:555–62.
10. Thiery JP. Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer. 2002;2:442–54.
11. Vleminckx K, Vakaet Jr L, Mareel M, Fiers W, van Roy F. Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasion suppressor role. Cell. 1991;66:107–19.
12. Blanco MJ, Moreno-Bueno G, Sarrio D, Locascio A, Cano A, Palacios J, *et al.* Correlation of snail expression with histological

grade and lymph node status in breast carcinomas. Oncogene. 2002;21:3241–6.
13. Peinado H, Ballestar E, Esteller M, Cano A. Snail mediates E-cadherin repression by the recruitment of the Sin3A/histone deacetylase 1 (HDAC1)/HDAC2 complex. Mol Cell Biol. 2004;24:306–19.
14. Huber MA, Kraut N, Beug H. Molecular requirements for epithelial-mesenchymal transition during tumor progression. Curr Opin Cell Biol. 2005;17:548–58.
15. Thiery JP, Sleeman JP. Complex networks orchestrate epithelial-mesenchymal transitions. Nat Rev Mol Cell Biol. 2006;7:131–42.
16. Zondag GC, Evers EE, ten Klooster JP, Janssen L, van der Kammen RA, Collard JG. Oncogenic Ras downregulates Rac activity, which leads to increased Rho activity and epithelial-mesenchymal transition. J Cell Biol. 2000;149:775–82.
17. Lo HW, Tsu SC, Xia W, Cao X, Shih JY, Wei Y, *et al.* Epidermal growth factor receptor cooperates with signal transducer and activator of transcription 3 to induce epithelial-mesenchymal transition in cancer cells via up-regulation of TWIST gene expression. Cancer Res. 2007;67:9066–76.
18. Smit MA, Geiger TR, Song JY, Gitelman I, Peeper DS. A Twist-Snail axis critical for TrkB-induced epithelial-mesenchymal transition-like transformation, anoikis resistance, and metastasis. Mol Cell Biol. 2009;29:3722–37.
19. Arumugam T, Ramachandran V, Fournier KF, Wang H, Marquis L, Abbruzzese JL, *et al.* Epithelial to mesenchymal transition contributes to drug resistance in pancreatic cancer. Cancer Res. 2009;69:5820–8.
20. Derksen PW, Liu X, Saridin F, van der Gulden H, Zevenhoven J, Evers B, *et al.* Somatic inactivation of E-cadherin and p53 in mice leads to metastatic lobular mammary carcinoma through induction of anoikis resistance and angiogenesis. Cancer Cell. 2006;10:437–49.
21. Berezovskaya O, Schimmer AD, Glinskii AB, Pinilla C, Hoffman RM, Reed JC, *et al.* Increased expression of apoptosis inhibitor protein XIAP contributes to anoikis resistance of circulating human prostate cancer metastasis precursor cells. Cancer Res. 2005;65:2378–86.
22. Duxbury MS, Ito H, Zinner MJ, Ashley SW, Whang EE. CEACAM6 gene silencing impairs anoikis resistance and *in vivo* metastatic ability of pancreatic adenocarcinoma cells. Oncogene. 2004;23:465–73.
23. Lemmens VE, Janssen-Heijnen ML, Verheij CD, Houterman S, Repelaer van Driel OJ, Coebergh JW. Co-morbidity leads to altered treatment and worse survival of elderly patients with colorectal cancer. Br J Surg. 2005;92:615–23.
24. Zimmermann GR, Lehar J, Keith CT. Multi-target therapeutics: when the whole is greater than the sum of the parts. Drug Discovery Today. 2007;12:34–42.
25. Montgomery AM, Reisfeld RA, Cheresh DA. Integrin alpha v beta 3 rescues melanoma cells from apoptosis in three-dimensional dermal collagen. Proc Nat Acad Sci. 1994;91:8856–60.
26. Goldstein NB, Johannes WU, Gadeliya AV, Green MR, Fujita M, Norris DA, *et al.* Active N-Ras and B-Raf inhibit anoikis by down-regulating Bim expression in melanocytic cells. J Invest Dermatol. 2009;129:432–7.
27. Solit DB, Garraway LA, Pratilas CA, Sawai A, Getz G, Basso A, *et al.* BRAF mutation predicts sensitivity to MEK inhibition. Nature. 2006;439:358–62.
28. Zhang Y, Yang M, Ji Q, Fan D, Peng H, Yang C, *et al.* Anoikis induction and metastasis suppression by a new integrin alphavbeta3 inhibitor in human melanoma cell line M21. Invest New Drugs. 2011;29:666–73.
29. Stockwell S. FDA approval for vemurafenib & companion diagnostic test for late-stage melanoma. Oncology Times. August 2011.
30. Dummer R, Robert CP, Chapman PB, Sosman JA, Middleton M, Bastholt L, *et al.* AZD6244 (ARRY-142886) *vs* temozolomide (TMZ) in patients (pts) with advanced melanoma: an open-label, randomized, multicenter, phase II study. J Clin Oncol. 2008; 26: abs # 9033).
31. Banerji U, Camidge DR, Verheul HM, Agarwal R, Sarker D, Kaye SB, *et al.* The first-in-human study of the hydrogen sulfate (Hyd-sulfate) capsule of the MEK1/2 inhibitor AZD6244 (ARRY-142886): a phase I open-label multicenter trial in patients with advanced cancer. Clin Cancer Res. 2010;16:1613–23.
32. Grossman SA, Ye X, Piantadosi S, Desideri S, Nabors LB, Rosenfeld M, *et al.* Survival of patients with newly diagnosed glioblastoma treated with radiation and temozolomide in research studies in the United States. Clin Cancer Res. 2010;16:2443–9.
33. Bellail AC, Hunter SB, Brat DJ, Tan C, Van Meir EG. Microregional extracellular matrix heterogeneity in brain modulates glioma cell invasion. Int J Biochem Cell Biol. 2004;36:1046–69.
34. Reardon DA, Nabors LB, Stupp R, Mikkelsen T. Cilengitide: an integrin-targeting arginine-glycine-aspartic acid peptide with promising activity for glioblastoma multiforme. Expert Opin Invest Drugs. 2008;17:1225–35.
35. Paolillo M, Russo MA, Serra M, Colombo L, Schinelli S. Small molecule integrin antagonists in cancer therapy. Mini Rev Med Chem. 2009;9:1439–46.
36. Salmaggi A, Boiardi A, Gelati M, Russo A, Calatozzolo C, Ciusani E, *et al.* Glioblastoma-derived tumorospheres identify a population of tumor stem-like cells with angiogenic potential and enhanced multidrug resistance phenotype. Glia. 2006;54:850–60.
37. Farber K, Synowitz M, Zahn G, Vossmeyer D, Stragies R, van Rooijen N, *et al.* An alpha5beta1 integrin inhibitor attenuates glioma growth. Mol Cell Neurosci. 2008;39:579–85.
38. Attwell S, Roskelley C, Dedhar S. The integrin-linked kinase (ILK) suppresses anoikis. Oncogene. 2000;19:3811–5.
39. Edwards LA, Thiessen B, Dragowska WH, Daynard T, Bally MB, Dedhar S. Inhibition of ILK in PTEN-mutant human glioblastomas inhibits PKB/Akt activation, induces apoptosis, and delays tumor growth. Oncogene. 2005;24:3596–605.
40. Koul D, Shen R, Bergh S, Lu Y, de Groot JF, Liu TJ, *et al.* Targeting integrin-linked kinase inhibits Akt signaling pathways and decreases tumor progression of human glioblastoma. Mol Cancer Ther. 2005;4:1681–8.
41. Howlader N, Noone AM, Krapcho M, Neyman N, Aminou R, Waldron W, *et al.* (eds). SEER Cancer Statistics Review, 1975–2008, National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2008/, based on November 2010 SEER data submission, posted to the SEER web site, 2011.
42. Balan V, Nangia-Makker P, Schwartz AG, Jung YS, Tait L, Hogan V, *et al.* Racial disparity in breast cancer and functional germ line mutation in galectin-3 (rs4644): a pilot study. Cancer Res. 2008;68:10045–50.
43. Raz A, Pazerini G, Carmi P. Identification of the metastasis-associated, galactoside-binding lectin as a chimeric gene product with homology to an IgE-binding protein. Cancer Res. 1989;49:3489–93.
44. Song YK, Billiar TR, Lee YJ. Role of galectin-3 in breast cancer metastasis: involvement of nitric oxide. Am J Pathol. 2002;160:1069–75.
45. Konstantinov KN, Robbins BA, Liu FT. Galectin-3, a beta-galactoside-binding animal lectin, is a marker of anaplastic large-cell lymphoma. Am J Pathol. 1996;148:25–30.
46. Akahani S, Nangia-Makker P, Inohara H, Kim HR, Raz A. Galectin-3: a novel antiapoptotic molecule with a functional BH1 (NWGR) domain of Bcl-2 family. Cancer Res. 1997;57:5272–6.

47. Kim HR, Lin HM, Biliran H, Raz A. Cell cycle arrest and inhibition of anoikis by galectin-3 in human breast epithelial cells. Cancer Res. 1999;59:4148–54.
48. Nangia-Makker P, Hogan V, Honjo Y, Baccarini S, Tait L, Bresalier R, *et al.* Inhibition of human cancer cell growth and metastasis in nude mice by oral intake of modified citrus pectin. J Nat Cancer Inst. 2002;94:1854–62.
49. Lee E, Nichols P, Spicer D, Groshen S, Yu MC, Lee AS. GRP78 as a novel predictor of responsiveness to chemotherapy in breast cancer. Cancer Res. 2006;66:7849–53.
50. Lee AS. The glucose-regulated proteins: stress induction and clinical applications. Trends Biochem Sci. 2001;26:504–10.
51. Koumenis C. ER stress, hypoxia tolerance and tumor progression. Curr Mol Med. 2006;6:55–69.
52. Fernandez PM, Tabbara SO, Jacobs LK, Manning FCR, Tsangaris TN, Shwartz AM, *et al.* Overexpression of the glucose-regulated stress gene GRP78 in malignant but not benign human breast lesions. Breast Cancer Res Treat. 2000;59:15–26.
53. Li J, Lee AS. Stress induction of GRP78/BiP and its role in cancer. Curr Mol Med. 2006;6:45–54.
54. Fu Y, Lee AS. Glucose regulated proteins in cancer progression, drug resistance and immunotherapy. Cancer Biol Ther. 2006;5:741–4.
55. Dong D, Ko B, Baumeister P, Swenson S, Costa F, Markland F, *et al.* Vascular targeting and antiangiogenesis agents induce drug resistance effector GRP78 within the tumor microenvironment. Cancer Res. 2005;65:5785–91.
56. Shani G, Fischer WH, Justice NJ, Kelber JA, Vale W, Gray PC. GRP78 and Cripto form a complex at the cell surface and collaborate to inhibit transforming growth factor beta signaling and enhance cell growth. Mol Cell Biol. 2008;28:666–77.
57. Luo T, Wang J, Yin Y, Hua H, Jing J, Sun X, *et al.* (−)-Epigallocatechin gallate sensitizes breast cancer cells to paclitaxel in a murine model of breast carcinoma. Breast Cancer Res. 2010;12:R8.
58. Hazlehurst LA, Valkov N, Wisner L, Storey JA, Boulware D, Sullivan DM, *et al.* Reduction in drug-induced DNA double-strand breaks associated with beta1 integrin-mediated adhesion correlates with drug resistance in U937 cells. Blood. 2001;98:1897–903.
59. Pongrakhananon V, Nimmannit U, Luanpitpong S, Rojanasakul Y, Chanvorachote P. Curcumin sensitizes non-small cell lung cancer cell anoikis through reactive oxygen species-mediated Bcl-2 downregulation. Apoptosis. 2010;15:574–85.
60. Chen HW, Yu SL, Chen JJ, Li HN, Lin YC, Yao PL, *et al.* Anti-invasive gene expression profile of curcumin in lung adenocarcinoma based on a high throughput microarray analysis. Mol Pharmacol. 2004;65:99–110.
61. Limtrakul P, Chearwae W, Shukla S, Phisalphong C, Ambudkar SV. Modulation of function of three ABC drug transporters, P-glycoprotein (ABCB1), mitoxantrone resistance protein (ABCG2) and multidrug resistance protein 1 (ABCC1) by tetrahydrocurcumin, a major metabolite of curcumin. Mol Cell Biochem. 2007;296:85–95.
62. Plath T, Detjen K, Welzel M, von Marschall Z, Murphy D, Schirner M, *et al.* A novel function for the tumor suppressor p16(INK4a): induction of anoikis via upregulation of the alpha (5)beta(1) fibronectin receptor. J Cell Biol. 2000;150:1467–78.
63. Kiyono T, Foster SA, Koop JI, McDougall JK, Galloway DA, Klingelhutz AJ. Both Rb/p16INK4a inactivation and telomerase activity are required to immortalize human epithelial cells. Nature. 1998;396:84–8.
64. Hosotani R, Miyamoto Y, Fujimoto K, Doi R, Otaka A, Fujii N, *et al.* Trojan p16 peptide suppresses pancreatic cancer growth and prolongs survival in mice. Clin Cancer Res. 2002;8:1271–6.
65. Demers MJ, Thibodeau S, Noel D, Fujita N, Tsuruo T, Gauthier R, *et al.* Intestinal epithelial cancer cell anoikis resistance: EGFR-mediated sustained activation of Src overrides Fak-dependent signaling to MEK/Erk and/or PI3-K/Akt-1. J Cell Biochem. 2009;107:639–54.
66. Douma S, Van Laar T, Zevenhoven J, Meuwissen R, Van Garderen E, Peeper DS. Suppression of anoikis and induction of metastasis by the neurotrophic receptor TrkB. Nature. 2004;430:1034–9.
67. Zhao W, Wen W, Zhang Z, Liao Z, Zhang S, Huang G. Expression and significance of tyrosine kinase receptors B in nasopharyngeal carcinoma patients. Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi. 2007;21:497–500.
68. Ng YK, Wong EY, Lau CP, Chan JP, Wong SC, Chan AS, *et al.* K252a induces anoikis-sensitization with suppression of cellular migration in Epstein-Barr Virus (EBV)-associated nasopharyngeal carcinoma cells. Invest New Drugs. 2010 (in-press).
69. Cazorla M, Jouvenceau A, Rose C, Guilloux JP, Pilon C, Dranovsky A, *et al.* Cyclotraxin-B, the first highly potent and selective TrkB inhibitor, has anxiolytic properties in mice. PLoS ONE. 2010;5:e9777.
70. Thress K, Macintyre T, Wang H, Whitston D, Liu ZY, Hoffmann E, *et al.* Identification and preclinical characterization of AZ-23, a novel, selective, and orally bioavailable inhibitor of the Trk kinase pathway. Mol Cancer Ther. 2009;8:1818–27.
71. Ng YP, Cheung ZH, Ip NY. STAT3 as a downstream mediator of Trk signaling and functions. J Biol Chem. 2006;281:15636–44.
72. Grandis JR, Drenning SD, Zeng Q, Watkins SC, Melhem MF, Endo S, *et al.* Constitutive activation of Stat3 signaling abrogates apoptosis in squamous cell carcinogenesis *in vivo*. Proc Natl Acad Sci U S A. 2000;97:4227–32.
73. Hsiao JR, Jin YT, Tsai ST, Shiau AL, Wu CL, Su WC. Constitutive activation of STAT3 and STAT5 is present in the majority of nasopharyngeal carcinoma and correlates with better prognosis. Br J Cancer. 2003;89:344–9.
74. Neiva KG, Zhang Z, Miyazawa M, Warner KA, Karl E, Nor JE. Cross talk initiated by endothelial cells enhances migration and inhibits anoikis of squamous cell carcinoma cells through STAT3/Akt/ERK signaling. Neoplasia. 2009;11:583–93.
75. Lui VW, Yau DM, Wong EY, Ng YK, Lau CP, Ho Y, *et al.* Cucurbitacin I elicits anoikis sensitization, inhibits cellular invasion and *in vivo* tumor formation ability of nasopharyngeal carcinoma cells. Carcinogenesis. 2009;30:2085–94.
76. Swan EA, Jasser SA, Holsinger FC, Doan D, Bucana C, Myers JN. Acquisition of anoikis resistance is a critical step in the progression of oral tongue cancer. Oral Oncol. 2003;39:648–55.
77. Chung CH, Ely K, McGavran L, Varella-Garcia M, Parker J, Parker N, *et al.* Increased epidermal growth factor receptor gene copy number is associated with poor prognosis in head and neck squamous cell carcinomas. J Clin Oncol. 2006;24:4170–6.
78. Sok JC, Coppelli FM, Thomas SM, Lango MN, Xi S, Hunt JL, *et al.* Mutant epidermal growth factor receptor (EGFRvIII) contributes to head and neck cancer growth and resistance to EGFR targeting. Clin Cancer Res. 2006;12:5064–73.
79. Frederick BA, Helfrich BA, Coldren CD, Zheng D, Chan D, Bunn PA, *et al.* Epithelial to mesenchymal transition predicts gefitinib resistance in cell lines of head and neck squamous cell carcinoma and non-small cell lung carcinoma. Mol Cancer Ther. 2007;6:1683–91.
80. Zeng Q, Chen S, You Z, Yang F, Carey TE, Saims D, *et al.* Hepatocyte growth factor inhibits anoikis in head and neck squamous cell carcinoma cells by activation of ERK and Akt signaling independent of NFkappa B. J Biol Chem. 2002;277:25203–8.
81. van Oijen MG, Rijksen G, ten Broek FW, Slootweg PJ. Over-expression of c-Src in areas of hyper-proliferation in head and neck cancer, premalignant lesions and benign mucosal disorders. J Oral Pathol Med. 1998;27:147–52.
82. Sen B, Peng S, Saigal B, Williams MD, Johnson FM. Distinct interactions between c-Src and c-Met in mediating resistance to c-Src inhibition in head and neck cancer. Clin Cancer Res. 2011;17:514–24.

83. Brooks HD, Glisson BS, Bekele BN, Ginsberg LE, El-Naggar A, Culotta KS, *et al.* Phase 2 study of dasatinib in the treatment of head and neck squamous cell carcinoma. Cancer. 2011;117:2112–9.
84. Xu H, Stabile LP, Gubish CT, Gooding WE, Grandis JR, Siegfried JM. Dual blockade of EGFR and c-Met abrogates redundant signaling and proliferation in head and neck carcinoma cells. Clin Cancer Res. 2011;17:4425–38.
85. Zeng Q, McCauley LK, Wang CY. Hepatocyte growth factor inhibits anoikis by induction of activator protein 1-dependent cyclooxygenase-2. Implication in head and neck squamous cell carcinoma progression. J Biol Chem. 2002;277:50137–42.
86. Greenhough A, Smartt HJ, Moore AE, Roberts HR, Williams AC, Paraskeva C, *et al.* The COX-2/PGE2 pathway: key roles in the hallmarks of cancer and adaptation to the tumor microenvironment. Carcinogenesis. 2009;30:377–86.
87. Kao J, Genden EM, Chen CT, Rivera M, Tong CC, Misiukiewicz K, *et al.* Phase 1 trial of concurrent erlotinib, celecoxib, and reirradiation for recurrent head and neck cancer. Cancer. 2011;117:3173–81.
88. Kumar P, Yadav A, Patel SN, Islam M, Pan Q, Merajver SD, *et al.* Tetrathiomolybdate inhibits head and neck cancer metastasis by decreasing tumor cell motility, invasiveness and by promoting tumor cell anoikis. Mol Cancer. 2010;9:206.
89. Mukohara T, Engelman JA, Hanna NH, Yeap BY, Kobayashi S, Lindeman N, *et al.* Differential effects of gefitinib and cetuximab on non-small-cell lung cancers bearing epidermal growth factor receptor mutations. J Natl Cancer Inst. 2005;97:1185–94.
90. Tsao MS, Sakurada A, Cutz JC, Zhu CQ, Kamel-Reid S, Squire J, *et al.* Erlotinib in lung cancer-molecular and clinical predictors of outcome. N Engl J Med. 2005;353:133–44.
91. Lei QY, Wang LY, Dai ZY, Zha XL. The relationship between PTEN expression and anoikis in human lung carcinoma cell lines. Sheng Wu HuaXue Yu Sheng Wu Wu Li Xue Bao (Shanghai). 2002;34:463–8.
92. Bao B, Ali S, Kong D, Sarkar SH, Wang Z, Banerjee S, *et al.* Anti-tumor activity of a novel compound-CDF is mediated by regulating miR-21, miR-200, and PTEN in pancreatic cancer. PLoS ONE. 2011;6:e17850.
93. Lee SY, Hur GY, Jung KH, Jung HC, Lee SY, Kim JH, *et al.* PPAR-gamma agonist increase gefitinib's antitumor activity through PTEN expression. Lung Cancer. 2006;51:297–301.
94. Thomson S, Petti F, Sujka-Kwok I, Epstein D, Haley JD. Kinase switching in mesenchymal-like non-small cell lung cancer lines contributes to EGFR inhibitor resistance through pathway redundancy. Clin Exp Metastasis. 2008;25:843–54.
95. Wei L, Yang Y, Zhang X, Yu Q. Altered regulation of Src upon cell detachment protects human lung adenocarcinoma cells from anoikis. Oncogene. 2004;23:9052–61.
96. Altorki N, Lane ME, Bauer T, Lee PC, Guarino MJ, Pass H, *et al.* Phase II proof-of-concept study of pazopanib monotherapy in treatment-naive patients with stage I/II resectable non-small-cell lung cancer. J Clin Oncol. 2010;28:3131–7.
97. Wang X, Hawk N, Yue P, Kauh J, Ramalingam SS, Fu H, *et al.* Overcoming mTOR inhibition-induced paradoxical activation of survival signaling pathways enhances mTOR inhibitors' anticancer efficacy. Cancer Biol Ther. 2008;7:1952–8.
98. Engelman JA, Chen L, Tan X, Crosby K, Guimaraes AR, Upadhyay R, *et al.* Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. Nat Med. 2008;14:1351–6.
99. Yoon YK, Kim HP, Han SW, Oh do Y, Im SA, Bang YJ, *et al.* KRAS mutant lung cancer cells are differentially responsive to MEK inhibitor due to AKT or STAT3 activation: implication for combinatorial approach. Mol Carcinog. 2010;49:353–62.
100. Dowlati A, Kluge A, Nethery D, Halmos B, Kern JA. SCH66336, inhibitor of protein farnesylation, blocks signal transducer and activators of transcription 3 signaling in lung cancer and interacts with a small molecule inhibitor of epidermal growth factor receptor/human epidermal growth factor receptor 2. Anticancer Drugs. 2008;19:9–16.
101. Weerasinghe P, Garcia GE, Zhu Q, Yuan P, Feng L, Mao L, *et al.* Inhibition of Stat3 activation and tumor growth suppression of non-small cell lung cancer by G-quartet oligonucleotides. Int J Oncol. 2007;31:129–36.
102. Brozovic A, Majhen D, Roje V, Mikac N, Jakopec S, Fritz G, *et al.* Alpha(v)beta(3) Integrin-mediated drug resistance in human laryngeal carcinoma cells is caused by glutathione-dependent elimination of drug-induced reactive oxidative species. Mol Pharmacol. 2008;74:298–306.
103. Chien Y, White MA. RAL GTPases are linchpin modulators of human tumor-cell proliferation and survival. EMBO Reports. 2003;4:800–6.
104. Singhal SS, Sehrawat A, Mehta A, Sahu M, Awasthi S. Functional reconstitution of RLIP76 catalyzing ATP-dependent transport of glutathione-conjugates. Int J Oncol. 2009;34:191–9.
105. Singhal SS, Wickramarachchi D, Yadav S, Singhal J, Leake K, Vatsyayan R, *et al.* Glutathione-conjugate transport by RLIP76 is required for clathrin-dependent endocytosis and chemical carcinogenesis. Mol Cancer Ther. 2011;10:16–28.
106. Singhal SS, Sehrawat A, Sahu M, Singhal P, Vatsyayan R, Lelsani P, *et al.* RLIP76 transports sunitinib and sorafenib and mediates drug resistance in kidney cancer. Int J Cancer. 126: 1327–38.
107. Wu Z, Owens C, Chandra N, Popovic K, Conaway M, Theodorescu D. RalBP1 is necessary for metastasis of human cancer cell lines. Neoplasia. 2010;12:1003–12.
108. Singhal J, Nagaprashantha LD, Vatsyayan R, Awasthi S, Singhal SS. RLIP76, a glutathione-conjugate transporter, plays a major role in the pathogenesis of metabolic syndrome. PLoS ONE. 2011;6(9):e24688.
109. Anisimov VN, Piskunova TS, Popovich IG, Zabezhinski MA, Tyndyk ML, Egormin PA, *et al.* Gender differences in metformin effect on aging, life span and spontaneous tumorigenesis in 129/Sv mice. Aging. 2010;2:945–58.
110. Campos AC, Molognoni F, Melo FH, Galdieri LC, Carneiro CR, D'Almeida V, *et al.* Oxidative stress modulates DNA methylation during melanocyte anchorage blockade associated with malignant transformation. Neoplasia. 2007;9:1111–21.
111. Festuccia C, Gravina GL, D'Alessandro AM, Muzi P, Millimaggi D, Dolo V, *et al.* Azacitidine improves antitumor effects of docetaxel and cisplatin in aggressive prostate cancer models. Endocr Relat Cancer. 2009;16:401–13.
112. Fang MZ, Wang Y, Ai N, Hou Z, Sun Y, Lu H, *et al.* Tea polyphenol (−)-epigallocatechin-3-gallate inhibits DNA methyltransferase and reactivates methylation-silenced genes in cancer cell lines. Cancer Res. 2003;63:7563–70.
113. Muscella A, Greco S, Elia MG, Storelli C, Marsigliante S. Angiotensin II stimulation of Na+/K+ATPase activity and cell growth by calcium-independent pathway in MCF-7 breast cancer cells. J Endocrinol. 2002;173:315–23.
114. Rajasekaran SA, Ball WJ, Bander NH, Liu H, Pardee JD, Rajasekaran AK. Reduced expression of beta-subunit of Na, K-ATPase in human clear-cell renal cell carcinoma. J Urol. 1999;162:574–80.
115. Mijatovic T, Roland I, Van Quaquebeke E, Nilsson B, Mathieu A, Van Vynckt F, *et al.* The alpha1 subunit of the sodium pump could represent a novel target to combat non-small cell lung cancers. J Pathol. 2007;212:170–9.
116. Horisberger JD. Recent insights into the structure and mechanism of the sodium pump. Physiology. 2004;19:377–87.
117. Simpson CD, Mawji IA, Anyiwe K, Williams MA, Wang X, Venugopal AL, *et al.* Inhibition of the sodium potassium adenosine

triphosphatase pump sensitizes cancer cells to anoikis and prevents distant tumor formation. Cancer Res. 2009;69:2739–47.
118. Yan SR, Joseph RR, Rosen K, Reginato MJ, Jackson A, Allaire N, *et al.* Activation of NF-kappaB following detachment delays apoptosis in intestinal epithelial cells. Oncogene. 2005;24:6482–91.
119. Mijatovic T, Op De Beeck A, Van Quaquebeke E, Dewelle J, Darro F, de Launoit Y, *et al.* The cardenolide UNBS1450 is able to deactivate nuclear factor kappaB-mediated cytoprotective effects in human non-small cell lung cancer cells. Mol Cancer Ther. 2006;5:391–9.
120. Skinner MA, Wildeman AG. beta(1) integrin binds the 16-kDa subunit of vacuolar H(+)-ATPase at a site important for human papillomavirus E5 and platelet-derived growth factor signaling. J Biol Chem. 1999;274:23119–27.
121. Supino R, Scovassi AI, Croce AC, Dal Bo L, Favini E, Corbelli A, *et al.* Biological effects of a new vacuolar-H,-ATPase inhibitor in colon carcinoma cell lines. Ann N Y Acad Sci. 2009;1171:606–16.
122. Buhaescu I, Izzedine H. Mevalonate pathway: a review of clinical and therapeutical implications. Clin Biochem. 2007;40:575–84.
123. Oades GM, Senaratne SG, Clarke IA, Kirby RS, Colston KW. Nitrogen containing bisphosphonates induce apoptosis and inhibit the mevalonate pathway, impairing Ras membrane localization in prostate cancer cells. J Urol. 2003;170:246–52.
124. Saeki T, Sasaki Y, Itoh T. Zoledronate (ZOL): phase I and pharmacokinetics (PK)/pharmacodynamics (PD) study in cancer patients. Bone. 2000;26:41S.
125. Kubista B, Trieb K, Sevelda F, Toma C, Arrich F, Heffeter P, *et al.* Anticancer effects of zoledronic acid against human osteosarcoma cells. J Orthop Res. 2006;24:1145–52.
126. Roukos DH. Personalized cancer diagnostics and therapeutics. Expert Rev Mol Diagn. 2009;9:227–9.
127. Gascoigne KE, Taylor SS. Cancer cells display profound intra- and interline variation following prolonged exposure to antimitotic drugs. Cancer Cell. 2008;14:111–22.
128. Barabasi AL, Gulbahce N, Loscalzo J. Network medicine: a network-based approach to human disease. Nat Rev Genet. 2011;12:56–68.
129. Bedrosian I, Faries MB, Guerry 4th D, Elenitsas R, Schuchter L, Mick R, *et al.* Incidence of sentinel node metastasis in patients with thin primary melanoma (≤ or = 1 mm) with vertical growth phase. Ann Surg Oncol. 2000;7:262–7.
130. Greene VR, Johnson MM, Grimm EA, Ellerhorst JA. Frequencies of NRAS and BRAF mutations increase from the radial to the vertical growth phase in cutaneous melanoma. J Invest Dermatol. 2009;129:1483–8.
131. Berens ME, Giese A. "…those left behind." Biology and oncology of invasive glioma cells. Neoplasia. 1999;1:208–19.
132. Viswanathan GA, Seto J, Patil S, Nudelman G, Sealfon SC. Getting started in biological pathway construction and analysis. PLoS Comput Biol. 2008;4:e16.
133. Nagaprashantha LD, Vatsyayan R, Singhal J, Fast S, Roby R, Awasthi S, *et al.* Anti-cancer effects of novel flavonoid vicenin-2 as a single agent and in synergistic combination with docetaxel in prostate cancer. Biochem Pharmacol. 2011;82:1100–1109.
134. Holbeck SL, Collins JM, Doroshow JH. Analysis of Food and Drug Administration-approved anticancer agents in the NCI60 panel of human tumor cell lines. Mol Cancer Ther. 2010;9:1451–60.
135. Cross J. DxS Ltd. Pharmacogenomics. 2008;9:463–7.
136. Allegra CJ, Jessup JM, Somerfield MR, Hamilton SR, Hammond EH, Hayes DF, *et al.* American Society of Clinical Oncology provisional clinical opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. J Clin Oncol. 2009;27:2091–6.