Synergistic activity of agents targeting growth factor receptors, CDKs and downstream signaling molecules in a panel of pancreatic cancer cell lines and the identification of antagonistic combinations: Implications for future clinical trials in pancreatic cancer

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Abstract. Pancreatic cancer is one of the most aggressive, heterogeneous and fatal type of human cancers for which more effective therapeutic agents are urgently needed. Here, we investigated the sensitivity of a panel of seven human pancreatic cancer cell lines (HPCCLs) to treatment with various tyrosine kinase inhibitors (TKIs), cyclin-dependent kinase (CDK) inhibitors, an inhibitor of STAT3 stattic, and a cytotoxic agent gemcitabine both as single agents and in combination. The membranous expression of various receptors and the effect of selected agents on cell cycle distribution, cell signaling pathways and migration was determined using flow cytometry, western blot analysis and scratch wound healing assays, respectively. While the expression of both HER-3 and HER-4 was low or negative, the expression of EGFR and HER2 was high or intermediate in all HPCCLs. Of all the agents examined, the CDK1/2/5/9 inhibitor, dinaciclib, was the most potent agent which inhibited the proliferation of all seven HPCCLs with IC50 values of ≤10 nM, followed by SRC targeting TKI dasatinib (IC50 of ≤258 nM), gemcitabine (IC50 of ≤330 nM), stattic (IC50 of ≤2 µM) and the irreversible pan-HER TKI afatinib (IC50 of ≤2.95 µM). Treatment with afatinib and dasatinib inhibited the ligand-induced phosphorylation of EGFR and SRC respectively. Statistically significant associations were found between HER2 expression and response to treatment with the ALK/IGF-IR/InsR inhibitor ceritinib and fibroblast growth factor receptor (FGFR)1/2/3 inhibitor AZD4547, HER3 and IGF-IR expression and their response to treatment with TKIs targeting HER family members (erlotinib and afatinib), and c-MET and ALK7 expression and their response to treatment with stattic. Interestingly, treatment with a combination of afatinib with dasatinib and gemcitabine with dasatinib resulted in synergistic tumor growth inhibition in all HPCCLs examined. In contrast, the combination of afatinib with dinaciclib was found to be antagonistic. Finally, the treatment with afatinib, dasatinib and gemcitabine with dasatinib resulted in synergistic tumor growth inhibition in all HPCCLs examined. In contrast, the combination of afatinib with dinaciclib was found to be antagonistic. Finally, the treatment with afatinib, dasatinib and gemcitabine with dasatinib resulted in synergistic tumor growth inhibition in all HPCCLs examined. In conclusion, the CDK1/2/5/9 inhibitor, dinaciclib, was the most potent agent which inhibited the proliferation of all seven HPCCLs with IC50 values of ≤10 nM, followed by SRC targeting TKI dasatinib (IC50 of ≤258 nM), gemcitabine (IC50 of ≤330 nM), stattic (IC50 of ≤2 µM) and the irreversible pan-HER TKI afatinib (IC50 of ≤2.95 µM). Treatment with afatinib and dasatinib inhibited the ligand-induced phosphorylation of EGFR and SRC respectively. Statistically significant associations were found between HER2 expression and response to treatment with the ALK/IGF-IR/InsR inhibitor ceritinib and fibroblast growth factor receptor (FGFR)1/2/3 inhibitor AZD4547, HER3 and IGF-IR expression and their response to treatment with TKIs targeting HER family members (erlotinib and afatinib), and c-MET and ALK7 expression and their response to treatment with stattic. Interestingly, treatment with a combination of afatinib with dasatinib and gemcitabine with dasatinib resulted in synergistic tumor growth inhibition in all HPCCLs examined. In contrast, the combination of afatinib with dinaciclib was found to be antagonistic. Finally, the treatment with afatinib, dasatinib and gemcitabine with dasatinib resulted in synergistic tumor growth inhibition in all HPCCLs examined. In conclusion, the CDK1/2/5/9 inhibitor, dinaciclib, irreversible pan-HER TKI afatinib and SRC targeting TKI dasatinib were most effective at inhibiting the proliferation and migration of HPCCLs and the combination of afatinib with dasatinib and gemcitabine with dasatinib led to synergistic tumor growth inhibition in all HPCCLs examined. Our results support further investigation on the therapeutic potential of these combinations in future clinical trials in pancreatic cancer.

Introduction

Pancreatic cancer is one of the deadliest types of human cancer. Globally, there were an estimated 458,918 new cases of pancreatic cancer and 432,242 pancreatic cancer deaths in 2018 (1,2). In the USA, there will be an estimated 57,600 new cases of pancreatic cancer and 47,050 pancreatic cancer-related
In recent years, both the heterogeneous nature of pancreatic cancer and its complex biology and tumor microenvironment have been associated with resistance to therapy (24-26). In our previous study, we demonstrated that the second generation pan-HER family blocker, afatinib, was more effective than the first generation EGFR TKIs erlotinib and gefitinib in inhibiting the growth of human pancreatic cancer cells (27). We also reported the superiority of afatinib when used in combination with the IGF-IR inhibitor NVP-AEW541 (15). The aim of this study was to investigate the sensitivity of a panel of human pancreatic cancer cell lines established from patients at different stages of the disease to treatment with agents targeting various growth factor receptors such as HER family members (afatinib and erlotinib), c-MET (crizotinib and capmatinib), ALK (ceritinib and brigatinib), IGF-IR (NVP-AEW742 and linsitinib), SRC family (dasatinib, bosutinib and ponatinib), CDK inhibitors (palbociclib and dinaciclib), STAT3 inhibitor (stattic) and cytotoxic agent (gemcitabine) both as single agents and/or in combination. We also investigated the association between the cell surface expression of various growth factor receptors and their response to treatment with the above agents. Furthermore, the effect of selected agents on the phosphorylation of HER receptors and subsequent downstream signaling molecules, cell cycle distribution and migration of human pancreatic cancer cell lines were determined.

Materials and methods

Tumor cell lines. A panel of seven human pancreatic cancer cell lines (HPCCls) was used in this study including BxPC-3, Capan-1, FA-6, Panc-1, Mia-Paca-2, Hs766T and CF-PAC-1. All cell lines were cultured routinely at 37°C in a humidified atmosphere (5% CO₂) as described previously (15,28). BxPC-3, Capan-1 and FA-6 were cultured in RPMI-1640 medium (Sigma-Aldrich; Merck KGaA), Panc-1, Mia-Paca-2 and Hs766T were cultured in Dulbecco's modified Eagle's medium (DME) (Merck) and CF-PAC-1 was cultured in Iscove's modified medium each supplemented with 10% fetal bovine serum (FBS) (heat inactivated) (Sigma-Aldrich; Merck KGaA), antibiotics penicillin (50 µg/ml) and streptomycin (50 µg/ml) and neomycin (50 µg/ml) (Sigma-Aldrich; Merck KGaA). RPMI-1640 and Iscove's modified Dulbecco's medium were supplemented with 2 and 8 mM L-glutamine (Sigma-Aldrich; Merck KGaA) respectively.

Tyrosine kinase inhibitors and antibodies. Gemcitabine and crizotinib were purchased from Healthcare at Home (Burlington, UK) and Tocris (Avonmouth, UK), respectively. Afatinib, NVP-AEW742, stattic, brigatinib, linsitinib, ceritinib, crizotinib, ponatinib, dasatinib, bosutinib, AZD4547, palbociclib, erlotinib and dinaciclib were all purchased from Selleckchem (Europe Ltd. UK). The antibodies for
Flow cytometry. The surface expression of various growth factor receptors on HPCCls was accessed by flow cytometry as described previously (27,30). Approximately 1x10^6 cells suspended in 2% FBS medium were added to 1.5 ml Eppendorf, centrifuged (254 x g for 3 min), washed once with cold PBS and incubated with or without 10 µg/ml of the primary antibody by rotation at 4°C for 1 h. Following that, the cells were washed thrice with 1 ml of cold PBS by centrifugation (254 x g for 3 min) and incubated with secondary antibody STAR9B (1:200 dilution) at 4°C for 1 h. Finally, the cells were washed thrice with cold PBS by centrifugation and re-suspended in 1 ml of FACS flow buffer (Becton Dickinson UK, Ltd., Oxford). FACS analysis was carried out using Cell Quest Pro software (Becton Dickinson, version 6.0). A minimum of 10,000 events were measured through excitation of argon laser at 488 nm using an FITC detector (525 nm) as part of the BD FACS Calibur Flow cytometer (BD Biosciences).

Growth inhibition studies. In order to determine the effect of various agents on the proliferation of HPCCls, sulforhodamine B (SRB; Sigma-Aldrich; Merck KGaA) colorimetric assay was used as described previously (27). Briefly, 5x10^4 cells/well were seeded in 100 µl of growth medium supplemented with 2% FBS in a 96-well plate and incubated at 37°C (in a humidified atmosphere in 5% CO₂). Following a 4-h incubation, ‘time zero’ plate (representing the number of cells prior to treatment) was fixed with 10% trichloroacetic acid (Fisher Scientific, Loughborough, UK) for 1 h at room temperature, washed thrice with tap water and left to air dry overnight. For other plates, 100 µl of doubling dilutions of agents were added to each well in triplicate and incubated at 37°C until the controls (medium only) became confluent. These plates were then fixed as mentioned above, stained with 0.04% (w/v) SRB in 1% acetic acid for 1 h, washed thoroughly with 1% acetic acid and left to air dry overnight. The stained cells were solubilized with 100 µl/well of 10 mM Tris-Base and the absorbance of each well was measured at 565 nm using an Epoch plate reader (Thermo Fisher Scientific, Inc.). Growth as a percentage of control was determined using the following formula:

\[
\% \text{ Cell Growth} = \frac{X - Y}{Z - Y} \times 100;
\]

where X is the absorbance of the drug-treated well at 565 nm, Y is the absorbance prior to treatment at 565 nm and Z is the absorbance of the untreated cells at 565 nm.

The 50% inhibitory concentration of each agent (IC₅₀) was calculated using the non-linear least squares curve fitting (four parameter analysis, log (inhibitor) vs response, variable slope) using Gen5 software (BioTeck, UK).

Cell cycle distribution analysis. The effect of selected agents including inhibitors of HER family members, CDK, SRC, STAT3 and cytotoxic agent on the cell cycle distribution of HPCCls was investigated using flow cytometry. Approximately, 0.5x10^6 cells/well were seeded in 5 ml of 2% FBS medium with or without drugs at IC₅₀ and incubated at 37°C until the control wells (no drugs) became almost confluent. Following that, the cells were harvested by trypsinization and pooled with their respective supernatants, washed once with cold PBS by centrifugation (264 x g for 4 min) and fixed with 70% ice-cold ethanol for minimum of 3 h at -20°C. The cells were collected by centrifugation (450 x g for 5 min) washed thrice with cold PBS and stained with Guava cell cycle reagent (Luminex). Cells were then run through a Guava EasyCyte™ flow cytometer (Luminex Corp.) where 5,000 events were recorded by excitation with a argon laser (488 nm) using Yellow-B fluorescence (583/26 nm) and analyzed using Incyte™ soft 3.3 (Luminex Corp.).

Determination of the combination index. The effect of selected agents on the growth of HPCCls when used in combination was assessed using SRB assay as described previously (27). For each combination, two agents (TKIs or cytotoxic agent) were mixed at their respective 4x IC₅₀ value (determined previously as a single agent) followed by eight doubling dilutions. Data analysis was performed using CalcuSyn software (Biosoft, UK) and interpreted as follow: <0.9=synergistic effect, 0.9-1.1=additive effect, >1.1=antagonistic effect.

Western blot analysis. The effect of various agents on downstream signaling molecules of BxPC-3 and Capan-1 cells was investigated using western blot analysis. Briefly, 0.5x10^6 cells/well were grown in 5 ml of 10% FBS RPMI-1640 medium in 6-well plate to near confluency. Cells were washed once with 5 ml of 0.5% FBS RPMI-1640 medium and incubated at 37°C with the desired drug at a final concentration of 400 nM (or no inhibitor/medium only as a negative control) in 5 ml of fresh 0.5% FBS RPMI-1640 medium for 1 h. After that, the cancer cells were incubated for a further 15 min with EGF, HB-EGF or IGF-II or no ligand. The cells were then washed once with PBS and lysed with 400 µl of preheated lysis buffer (Invitrogen; Thermo Fisher Scientific, Inc.) containing protease inhibitor cocktail (Sigma-Aldrich; Merck KGaA) and homogenized using 25x5/8” gauge needles in order to reduce its viscosity. Protein samples (30 µg) were separated on 4-12% Bis-Tris gel (Invitrogen; Thermo Fisher Scientific, Inc.) using the XCell II Surelock Mini-Cell system (Invitrogen; Thermo
Fisher Scientific, Inc.) and transferred onto Immobilon-FL PVDF membranes (Merck) using XCell II Mini-Cell Blot Module kit (Invitrogen; Thermo Fisher Scientific, Inc.). The PVDF membranes were probed with various antibodies at the manufacturer’s recommended dilutions and visualized using the LI-COR Image Studio software (version 1.x-2.x).

**Scratch wound healing assay.** The effect of selected agents on the migration of HPCCLs was investigated using scratch wound healing experiment. Briefly, 1x10⁶ cells/100 μl of 10% FBS medium per well were seeded in a corning CELLBIND™ 96-well clear flat bottom sterile plate (Sigma-Aldrich; Merck KGaA). Following 24 h of incubation at 37°C, wounds were created using a wound maker, carefully aspirating medium from each well. After washing with PBS, cells were treated with 200 μl of 10% FBS medium containing various drugs or medium only as positive control. The plate was then placed onto the IncuCyte Zoom® instrument at 37°C for 72 h where cells were analyzed every 3 h using Incucyte Zoom® software (Essen Bioscience, version 2018A).

**Statistical analysis.** The statistical analysis was carried out using SPSS software (IBM®, SPSS statistics version 26). Linear regression analysis was used to assess the relationship between the expression of HER family members and response to treatment with various TKIs, CDK inhibitor, STAT3 inhibitor and cytotoxic agent. The effect of selected agents on the migration of pancreatic cancer cell lines were tested by paired t-test analysis. A P-value of ≤0.05 was considered to be statistically significant and an R² value closer to 1 showed the reliability of the association between the IC₅₀ value of each drug and expression level of each marker. All statistical analyses were carried out using SPSS statistics 26 (SPSS Inc.).

**Results**

**Cell surface expression of various growth factor receptors in pancreatic cancer cell lines.** We determined the expression level of various membrane bound growth factor receptors including HER family members, IGF-IR, c-MET and ALK7 in seven HPCCLs using flow cytometry and the results are represented as mean fluorescence intensity (MFI) and histograms (Table I, Fig. 1). Most cell lines were found to have high expression for EGFR and moderate expression for HER2. In comparison to control EGFR-overexpressing breast cell line MDA-MB-468 (MFI=806), the MFI values for EGFR expression in our panel ranged from moderate (Mia-Paca-2, MFI=21.1) to high (Hs766T, MFI=236.9). Similarly, in comparison to control HER2-overexpressing ovarian cancer cell line SKOV3 (MFI=385), the MFI values for HER2 expression in our panel of HPCCLs ranged from low (Hs766T, MFI=11.2) to moderate (Capan-1, MFI=33.1). The expression level of HER3 and HER4 was undetectable to very low in most of the cell lines. Finally, in comparison to the IGF-IR and ALK7-positive breast cancer cell lines MDA-MB231 (MFI of 23 and 20, respectively) and c-MET positive MDA-MB468 (MFI=35), our panel of human pancreatic cancer cells had low to moderate level of expression with BxPC-3 cells having the highest expression of both IGFIR (MFI=28.4) and ALK (MFI=50.2) and Hs766T having the highest level of expression of C-MET with an MFI value of 50.8 respectively (Table I).

**Growth response of human pancreatic cancer cell lines to treatment with various TKIs, CDK inhibitors, STAT3 inhibitor and cytotoxic agent.** The effect of various agents on the growth of HPCCLs was determined using SRB assay and the results are expressed as IC₅₀ (Table II, Fig. 2). Of all the agents tested, the CDK1/2/5/9 inhibitor dinaciclib was the most potent agent inhibiting the growth of all seven cell lines with the IC₅₀ values of ≤10 nM. This was followed by the Abl/Src/c-kit TKI dasatinib with IC₅₀ values of 13 nM (CF-PAC1) to 258 nM (Panc-1); gemcitabine with IC₅₀ of ≤330 nM; the STAT3 inhibitor, static, with IC₅₀ values of 0.74 μM (Panc-1) to 2 μM (BxPC-3) and the irreversible pan-HER TKI afatinib with IC₅₀ of 27 nM (BxPC-3) to 2.95 μM (FA-6). The morphology of BxPC-3 cells following treatment with the above agents compared to the control are presented in Fig. 3. The effect of c-MET/ALK inhibitor, crizotinib, ALK/IGF-IR/InSr inhibitor ceritinib, Abl/PDGFRα/VEGFR2/FGFR1/Src inhibitor ponatinib and Src/Abl inhibitor, bosutinib, on the growth of all HPCCLs was found to be moderate (Table II). However, treatment with the PDGFRα/β inhibitor crenolanib, FGFR1/2/3 inhibitor AZD4547, c-MET inhibitor capmatinib, IGF-IR inhibitor NVP-AEW742, and IGF-IR/InSr inhibitor brigatinib had minimum to no inhibitory effect on HPCCLs (Table II).

**Cell cycle distribution analysis.** The effect of various agents on the cell cycle distribution of four HPCCLs was determined using flow cytometry and the results are summarized in Table III. Treatment with afatinib, dinaciclib, dasatinib, static and gemcitabine increased the percentage of cells in the sub-G1 phase (apoptotic/dead cells) with subsequent reduction in the G0/G1 phase. Furthermore, treatment with afatinib and dasatinib increased cells in the S and G2/M phase whereas treatment with dinaciclib and gemcitabine increased cells in the S-phase in most of the cell lines examined. The representative flow cytometry plots of cell cycle distribution of BxPC-3 cells following treatment with these agents is shown in Fig. S1.

**Afatinib and dasatinib blocks the phosphorylation of EGFR and SRC respectively.** Next we investigated the effects of treatment with afatinib, dinaciclib, dasatinib and static on the phosphorylation of growth factor receptors and downstream cell signaling molecules in BxPC-3 and Capan-1 cells. As expected, afatinib blocked the ligand-induced phosphorylation of tyrosine and phosphorylation of EGFR at position 1.068 which in turn resulted in reduction in the phosphorylation of downstream signaling molecules such as MAPK and AKT in both cell lines examined (BxPC-3 and Capan-1) (Fig. 4A and B). Treatment with dasatinib alone was accompanied by the EGFR, HB-EGF and IGF-II induced phosphorylation of SRC. However, no phosphorylation of the IGF-IR was evident following treatment with EGF, HB-EGF and IGF-II in these two cancer cell lines (data not shown).

**Synergistic and antagonistic effect of various drug combinations in pancreatic cancer cell lines.** The combined effect of various agents including afatinib, dinaciclib, dasatinib, static...
Table I. Surface expression of various growth factor receptors in human pancreatic cancer cell lines.

| Pancreatic cancer cell lines | Control | EGFR | HER2 | HER3 | HER4 | IGF-IR | C-MET | ALK7 |
|-----------------------------|---------|------|------|------|------|--------|-------|------|
| BxPC-3 (primary tumor)      | 4.1±0.3 | 195.6±36.1 | 22.5±4.5 | 12.7±1.3 | 11.1±0.2 | 28.4±2.2 | 40.5±5.3 | 50.2±9.7 |
| Capan-1 (liver metastasis)  | 5.3±0.4 | 63.6±6.8 | 33.12±3.2 | 8.4±0.5 | 7.5±0.4 | 22.3±0.2 | 23.5±0.9 | 24.3±0.6 |
| FA-6 (unknown)              | 4.1±0.5 | 133.6±11.5 | 13.9±0.3 | 8.67±0.1 | 6.48±0.5 | 9.1±0.4 | 17.2±0.5 | 24.0±0.3 |
| Panc-1 (primary tumor)      | 5.0±0.5 | 232.6±4.4 | 32.3±4.6 | 7.37±0.4 | 11.4±1.0 | 20.3±1.5 | 5.0±0.0 | 23.5±1.3 |
| Mia-Paca2 (primary tumor)   | 4.9±0.1 | 26.0±9.1 | 22.6±0.5 | 5.26±0.2 | 7.93±0.3 | 16.8±0.5 | 6.0±0.2 | 6.8±0.4 |
| Hs766T (lymph node metastasis) | 4.1±0.6 | 241±4.5 | 11.2±0.4 | 10.4±0.1 | 15.5±0.4 | 16.1±0.8 | 50.8±7.2 | 37.5±3.0 |
| CF-PAC1 (liver metastasis)  | 4.5±0.5 | 96.7±7.8 | 48.3±1.4 | 18.1±0.8 | 9.52±0.2 | 26.2±3.2 | 38.8±4.2 | 26.3±0.6 |

Controls

|          | EGFR | HER2 | HER3 | HER4 | IGF-IR | C-MET | ALK7 |
|----------|------|------|------|------|--------|-------|------|
| MDA-MB-468 | 4.4±0.3 | N/A | N/A | N/A | 35.3±1.7 | N/A |
| SKOV3 (ovarian) | N/A | 385.2±0.4 | N/A | N/A | N/A | N/A |
| MDA-MB-231 | 4.0±0.3 | N/A | 23.2±0.6 | N/A | 20.3±1.9 |

N/A, not available. MDA-MB-231, MBA-MB-468 and SKOV3 cell lines were used as positive controls. EGFR, epidermal growth factor receptor; HER, human epidermal growth factor receptor; IGF-IR, insulin-like growth factor 1 receptor.

Figure 1. The membrane bound expression level of various growth factor receptors determined by flow cytometry in human pancreatic cancer cell lines represented as histograms. EGFR, epidermal growth factor receptor; HER, human epidermal growth factor receptor; c-MET, mesenchymal-epithelial transition factor; IGF-IR, insulin-like growth factor 1 receptor; ALK7, anaplastic lymphoma kinase 7.
Table II. IC\textsubscript{50} values of various agents on HPCCLs as assessed by SRB colorimetric assay.

| Cell lines | Palbociclib (CDK4/6 inhibitor) | Dinacicilib (CDK1/2/5/9 inhibitor) | Crizotinib (c-MET/ALK TKI) | Capmatinib (c-MET TKI) | Ceritinib (ALK/IGF-IR/InsR TKI) | Brigatinib (ALK/ROS1 TKI) | Erlotinib (reversible EGFR TKI) | Afatinib (irreversible pan-HER TKI) | Gemcitabine (cytotoxic agent) |
|------------|---------------------------------|-------------------------------------|--------------------------|-----------------------|---------------------------------|---------------------------|-------------------------------|-------------------------------|-----------------------------|
| BxPC-3     | 3.87±1.58                       | 0.010±0.21                          | 1.83±0.30                | >10.00                | 1.43±0.17                       | >10.00                    | 1.70±0.05                     | 0.027±0.01                     | 0.003±0.02                   |
| Capan-1    | 2.24±0.24                       | 0.007±0.03                          | 1.74±0.06                | >10.00                | 1.39±0.01                       | 3.33±0.01                 | >10.00                        | 2.21±0.03                      | 0.33±0.00                   |
| FA-6       | 4.28±0.18                       | 0.005±0.15                          | 1.21±0.00                | >10.00                | 1.30±0.00                       | 5.30±0.02                 | >10.00                        | 2.95±0.04                      | 0.06±0.01                   |
| Panc-1     | >10.00                          | 0.010±0.02                          | 2.93±0.14                | >10.00                | 2.65±0.29                       | >10.00                    | >10.00                        | 2.63±0.50                      | 0.02±0.02                   |
| Mia-Paca2  | 6.90±0.14                       | 0.006±0.02                          | 1.73±0.10                | >10.00                | 1.33±0.02                       | >10.00                    | >10.00                        | 1.76±0.05                      | 0.003±0.00                   |
| Hs766T     | 4.31±0.41                       | 0.010±0.34                          | 3.13±0.22                | >10.00                | 1.55±0.03                       | >10.00                    | 7.7±0.04                      | 1.72±0.01                      | 0.008±0.01                   |
| CF-PAC1    | >10.00                          | 0.010±0.06                          | 3.29±0.20                | >10.00                | 5.02±0.20                       | >10.00                    | 0.96±0.12                     | 0.054±0.01                     | 0.06±0.00                    |

| Cell lines | Dasatinib (Abl/Src/c-kit TKI) | NVP-AEW742 (IGF-IR TKI) | Linsitinib (IGF-IR/InsR TKI) | Static (STAT3 inhibitor) | Cremolanib (PDGFRα/β KI) | Bosutinib (Src/Abl TKI) | AZD4547 (FGFR1/2/3 TKI) | Ponatinib (Abl/PDGFRα/ VEGFR2/FGFR1/Src TKI) |
|------------|---------------------------------|------------------------|---------------------------|------------------------|--------------------------|------------------------|------------------------|--------------------------------|
| BxPC-3     | 0.07±0.00                       | 6.74±0.31              | 5.43±0.22                 | 2.00±0.22              | 4.59±0.48                | 0.73±0.02              | 4.39±0.05               | 0.40±0.04                     |
| Capan-1    | 0.16±0.02                       | >10.00                 | 9.00±0.01                 | 0.75±0.06              | 3.54±0.03                | 0.98±0.03              | 5.86±0.19               | 0.35±0.02                     |
| FA-6       | 0.15±0.01                       | >10.00                 | 7.52±0.48                 | 0.77±0.06              | 2.51±0.09                | 1.5±0.10               | 2.51±0.01               | 0.33±0.02                     |
| Panc-1     | 0.258±0.03                      | >10.00                 | >10.00                    | 0.74±0.03              | 6.32±0.06                | 5.01±0.40              | >10.00                 | 4.85±0.13                     |
| Mia-Paca2  | 0.08±0.01                       | >10.00                 | 5.65±1.21                 | 0.80±0.12              | 3.08±1.03                | 3.81±0.11              | 4.72±0.02               | 2.52±0.06                     |
| Hs766T     | 0.21±0.05                       | >10.00                 | >10.00                    | 1.85±0.07              | 1.29±0.32                | 0.32±0.12              | 3.37±0.12               | 3.69±0.04                     |
| CF-PAC1    | 0.013±0.03                      | >10.00                 | >10.00                    | 1.51±0.12              | 3.49±0.11                | 1.13±0.16              | 9.59±0.30               | 0.51±0.02                     |

Each value represents mean IC\textsubscript{50} ± SD. IC\textsubscript{50}, 50% inhibitory concentration; HPCCLs, human pancreatic cancer cell lines; SRB, sulforhodamine B; TKI, tyrosine kinase inhibitors; CDK, cyclin-dependent kinase.
and gemcitabine on the growth of HPCCLs was investigated. Only treatment with a combination of dasatinib with afatinib or dasatinib in combination with gemcitabine led to synergistic growth inhibition of four HPCCLs (Table IV). In contrast, treatment with a combination of afatinib with dinaciclib was found to be antagonistic in all of four HPCCLs examined (Table IV). Finally, treatment with other drug combinations resulted in mixed effects in all cell lines examined (data not shown).

**Linear regression analysis.** The association between the expression level of various growth factor receptors and their response to treatment with various agents was assessed using SPSS software (Table V). There was no correlation between expression level of EGFR and the response to treatment with various agents. However, there were some statistically significant associations between HER2 expression and the response to treatment with ALK/IGF-IR/InsR inhibitor ceritinib ($R^2=0.698, P=0.019$) and the FGFR1/2/3 inhibitor AZD4547 ($R^2=0.751, P=0.012$); HER3 expression and the response to treatment with HER family targeting TKI erlotinib ($R^2=0.830, P=0.004$) and afatinib ($R^2=0.599, P=0.041$); IGF-IR expression and the response to treatment with erlotinib ($R^2=0.608, P=0.039$) and afatinib ($R^2=0.672, P=0.024$). In addition, a statistically significant association was found between c-MET expression and the response to treatment with STAT3 inhibitor stattic ($R^2=0.809, P=0.006$) and SRC/Abl inhibitor bosutinib ($R^2=0.747, P=0.012$) and finally between ALK7 expression and the response to treatment with STAT3 inhibitor stattic ($R^2=0.682, P=0.022$). HER4 was not tested due to its negative expression in all cell lines.

**Effect of selected agents on the migration of pancreatic cancer cell lines.** The effect of selected agents on the migration of four HPCCLs was determined using scratch wound healing assay and the results at time points 6, 12 and 24 h are summarized in Fig. 5A. As an example, the effect of these agents on the migration of BxPC-3 cells at 24 h is shown in Fig. 5B. In comparison to...
positive control (i.e., no treatment, 10% FBS medium only) most drugs inhibited the migration of HPCCLs with SRC targeting TKI dasatinib, and the CDK inhibitor dinaciclib and the irreversible pan-HER TKI afatinib being most effective (Fig. 5A and B).
Figure 4. Effect of afatinib, dinaciclib, dasatinib, stattic and NVP-AEW742 with or without ligands (EGF, HB-EGF, IGF-II) on the phosphorylation of EGFR and downstream cell signaling molecules including MAPK, AKT, STAT3, SRC and IGF-IR in BxPC-3 (A) and Capan-1 (B) cells. The cells were cultured in 10% FBS RPMI-1640 medium to near confluency. Cells were washed once with 0.5% FBS RPMI-1640 medium and incubated with selected agents (400 nM) for 1 h and then stimulated with 40 nM ligands (EGF, HB-EGF and IGF-II) for 15 min. Cells were then lysed, separated using SDS-PAGE, transferred onto PDVF membranes, probed with the antibodies of interest and visualized using LI-COR software. EGF, epidermal growth factor; HB-EGF, heparin-binding EGF-like growth factor; IGF-II, insulin-like growth factor II; EGFR, epidermal growth factor receptor; MAPK, mitogen-activated protein kinase; AKT, protein kinase B or PKB; STAT3, signal transducer and activator of transcription 3; SRC, proto-oncogene tyrosine kinase SRC; IGF-IR, insulin-like growth factor 1 receptor.
Table IV. Combination index (CI) values of dinaciclib plus afatinib, dasatinib plus afatinib and dasatinib plus gemcitabine in human pancreatic cancer cell lines.

| Cell lines  | Dinaciclib + afatinib combination index (effect) | Afatinib + dasatinib combination index (effect) | Dasatinib + gemcitabine combination index (effect) |
|-------------|-------------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| BxPC-3      | 1.32 (slight antagonism)                        | 0.97 (nearly additive)                        | 0.85 (moderate synergism)                      |
| Capan-1     | 1.85 (antagonism)                               | 0.39 (synergism)                              | 0.35 (synergy)                                |
| FA-6        | 1.60 (antagonism)                               | 0.53 (synergism)                              | 0.66 (synergy)                                |
| Mia-Paca2   | 1.66 (antagonism)                               | 0.58 (synergism)                              | 0.85 (moderate synergism)                      |

Discussion

Despite major advances in the early diagnosis and treatment of solid tumors in the past three decades, pancreatic cancer remains as one of the most aggressive and deadliest forms of cancer. By 2030, it is predicted to become the second leading cause of cancer-related deaths after lung cancer (31). Due to its heterogenous nature, its retroperitoneal location, non-specific symptoms and lack of screening methods, the overwhelming majority of pancreatic cancer patients are diagnosed at an advanced stage of the disease at the time of presentation (32). To date, of the HER inhibitors, only the EGFR-specific tyrosine kinase inhibitor (TKI), erlotinib has gained the FDA approval for the targeted therapy of locally advanced, unresectable or metastatic pancreatic cancer in combination with the cytotoxic agent gemcitabine (14). However, the majority of pancreatic cancer patients have either primary resistance, or develop secondary resistance following a short course of erlotinib. As a result, the duration of response can be short in many patients (7,30). Therefore, there is an urgent need to discover more effective therapeutic interventions for patients diagnosed with different stages of pancreatic cancer (7).

We previously demonstrated that of the pan HER family blocker, afatinib was more effective than erlotinib in inhibiting the growth of human pancreatic cancer cells (27). We also reported that treatment with afatinib in combination the insulin-like growth factor 1 receptor (IGF-IR) inhibitor NVP-AEW541 resulted in synergistic growth inhibition of pancreatic cancer cells (15). Due to the heterogeneous nature of pancreatic cancer, in this study we investigated the growth response of human pancreatic cancer cell lines (HPCCCLs), established from patients at different stages of the disease, to the treatment with agents targeting different cyclin-dependent kinases (CDKs), various growth factor receptors and cell signaling molecules.

Of all the agents examined, the CDK1/2/5/9 inhibitor, dinaciclib was the most potent agent and inhibited the proliferation of all seven primary and metastatic HPCCCLs with IC<sub>50</sub> values of ≤10 nM. Pre-clinical testing of dinaciclib exhibited an acceptable toxicity profile and effective inhibition in mouse models and it was found to be safe and well tolerated in phase I trials (33,34). The second most effective agent with anti-proliferative activity was the SRC/c-kit/Abl inhibitor dasatinib. It inhibited the growth of all seven primary and metastatic HPCCCLs with IC<sub>50</sub> of ≤258 nM. However, the most sensitive cell line to growth inhibition by dasatinib were Bx-PC3 and Mia-PaCa-2, which were established from two primary tumors and with IC<sub>50</sub> values of 70 and 80 nM, respectively. The third most effective agent was the STAT3 inhibitor stattic which inhibited the growth of all HPCCCLs with IC<sub>50</sub> of ≤2 µM. The pan-HER family blocker also inhibited the growth of all seven HPCCCLs with IC<sub>50</sub> values ≤2.95 µM. However, the most sensitive cell lines to treatment with afatinib were the EGFR, HER2 and HER3-positive Bx-PC-3 and CF-PAC1 cells whereas the reversible EGFR TKI erlotinib was only effective in Bx-PC-3, CF-PAC1 and Hs766T cells. We found a statistically significant association between HER3 expression and their response to treatment with TKIs targeting HER family members. In another study, Frolov and colleagues suggested that the higher sensitivity of HER3-positive cell lines to treatment with pan-HER TKIs could be due to blockade of HER-3 transactivation via EGFR therefore inhibiting PI3K/AKT signaling pathways (35). Other studies have also supported the association between the expression level of HER3 and the sensitivity of pancreatic cancer cell lines to treatment with erlotinib (35,36). Although our panel of cell lines was moderately positive for IGF-IR and showed moderate sensitivity to agents targeting IGF-IR and fibroblast growth factor receptor (FGFR), some statistically significant associations were found between IGF-IR expression and their response to treatment with HER family TKIs as well as HER2 expression and treatment with ALK/IGF-IR/InsR inhibitor ceritinib and FGFR inhibitor AZD4547. Both IGF-IR and FGFR-mediated signaling pathways have been found to participate in the resistance to anti-HER targeted therapy (15,37,38). Moreover, we and other researchers have shown that the co-targeting of IGF-IR and HER family members results in synergistic growth inhibition of human pancreatic cancer cells (15,39).

The synchronous activation of bypass pathways via receptor tyrosine kinases (RTKs) including c-MET and ALK7 has been shown to induce resistance to current treatments and have emerged as important therapeutic targets for pancreatic cancer treatments. The expression level of both these receptors in our panel of cell lines was moderate. While the growth of all seven HPCCCLs were inhibited by the dual c-MET/ALK TKI crizotinib (i.e., IC<sub>50</sub> values ≤2.3 × 10<sup>-6</sup> M) in our panel of cell lines was moderate. While the growth of all seven HPCCCLs were inhibited by the dual c-MET/ALK TKI crizotinib (i.e., IC<sub>50</sub> values ≤2.3 × 10<sup>-6</sup> M) in our panel of cell lines was moderate. While the growth of all seven HPCCCLs were inhibited by the dual c-MET/ALK TKI crizotinib (i.e., IC<sub>50</sub> values ≤2.3 × 10<sup>-6</sup> M) in our panel of cell lines was moderate. While the growth of all seven HPCCCLs were inhibited by the dual c-MET/ALK TKI crizotinib (i.e., IC<sub>50</sub> values ≤2.3 × 10<sup>-6</sup> M) in our panel of cell lines was moderate. While the growth of all seven HPCCCLs were inhibited by the dual c-MET/ALK TKI crizotinib (i.e., IC<sub>50</sub> values ≤2.3 × 10<sup>-6</sup> M) in our panel of cell lines was moderate. While the growth of all seven HPCCCLs were inhibited by the dual c-MET/ALK TKI crizotinib (i.e., IC<sub>50</sub> values ≤2.3 × 10<sup>-6</sup> M) in our panel of cell lines was moderate. While the growth of all seven HPCCCLs were inhibited by the dual c-MET/ALK TKI crizotinib (i.e., IC<sub>50</sub> values ≤2.3 × 10<sup>-6</sup> M) in our panel of cell lines was moderate. While the growth of all seven HPCCCLs were inhibited by the dual c-MET/ALK TKI crizotinib (i.e., IC<sub>50</sub> values ≤2.3 × 10<sup>-6</sup> M) in our panel of cell lines was moderate. While the growth of all seven HPCCCLs were inhibited by the dual c-MET/ALK TKI crizotinib (i.e., IC<sub>50</sub> values ≤2.3 × 10<sup>-6</sup> M) in our panel of cell lines was moderate. While the growth of all seven HPCCCLs were inhibited by the dual c-MET/ALK TKI crizotinib (i.e., IC<sub>50</sub> values ≤2.3 × 10<sup>-6</sup> M) in our panel of cell lines was moderate. While the growth of all seven HPCCCLs were inhibited by the dual c-MET/ALK TKI crizotinib (i.e., IC<sub>50</sub> values ≤2.3 × 10<sup>-6</sup> M) in our panel of cell lines was moderate. While the growth of all seven HPCCCLs were inhibited by the dual c-MET/ALK TKI crizotinib (i.e., IC<sub>50</sub> values ≤2.3 × 10<sup>-6</sup> M) in our panel of cell lines was moderate. While the growth of all seven HPCCCLs were inhibited by the dual c-MET/ALK TKI crizotinib (i.e., IC<sub>50</sub> values ≤2.3 × 10<sup>-6</sup> M) in our panel of cell lines was moderate. While the growth of all seven HPCCCLs were inhibited by the dual c-MET/ALK TKI crizotinib (i.e., IC<sub>50</sub> values ≤2.3 × 10<sup>-6</sup> M) in our panel of cell lines was moderate. While the growth of all seven HPCCCLs were inhibited by the dual c-MET/ALK TKI crizotinib (i.e., IC<sub>50</sub> values ≤2.3 × 10<sup>-6</sup> M) in our panel of cell lines was moderate. While the growth of all seven HPCCCLs were inhibited by the dual c-MET/ALK TKI crizotinib (i.e., IC<sub>50</sub> values ≤2.3 × 10<sup>-6</sup> M) in our panel of cell lines was moderate. While the growth of all seven HPCCCLs were inhibited by the dual c-MET/ALK TKI crizotinib (i.e., IC<sub>50</sub> values ≤2.3 × 10<sup>-6</sup> M) in our panel of cell lines was moderate. While the growth of all seven HPCCCLs were inhibited by the dual c-MET/ALK TKI crizotinib (i.e., IC<sub>50</sub> values ≤2.3 × 10<sup>-6</sup> M) in our panel of cell lines was moderate. While the growth of all seven HPCCCLs were inhibited by the dual c-MET/ALK TKI crizotinib (i.e., IC<sub>50</sub> values ≤2.3 × 10<sup>-6</sup> M) in our panel of cell lines was moderate. While the growth of all seven HPCCCLs were inhibited by the dual c-MET/ALK TKI crizotinib (i.e., IC<sub>50</sub> values ≤2.3 × 10<sup>-6</sup> M) in our panel of cell lines was moderate.
Table V. Linear regression analysis of the expression of various receptors against the sensitivity of human pancreatic cancer cell lines to treatment with various TKIs, CDK inhibitors, STAT3 inhibitor and a cytotoxic agent.

| Drugs/cell surface markers | EGFR R² (P-value) | HER2 R² (P-value) | HER3 R² (P-value) | IGF-IR R² (P-value) | C-MET R² (P-value) | ALK7 R² (P-value) |
|---------------------------|------------------|------------------|------------------|-------------------|------------------|------------------|
| Palbociclib               | 0.004 (0.895)    | 0.345 (0.165)    | 0.046 (0.646)    | 0.001 (0.940)     | 0.091 (0.511)    | 0.125 (0.437)    |
| Crizotinib                | 0.203 (0.310)    | 0.185 (0.336)    | 0.230 (0.276)    | 0.017 (0.779)     | 0.158 (0.377)    | 0.029 (0.715)    |
| Ceritinib                 | 0.001 (0.948)    | **0.698 (0.019)**| 0.496 (0.077)    | 0.038 (0.674)     | 0.031 (0.704)    | 0.002 (0.916)    |
| Brigatinib                | 0.184 (0.692)    | 0.180 (0.343)    | 0.132 (0.424)    | 0.069 (0.569)     | 0.031 (0.705)    | 0.021 (0.756)    |
| Erlotinib                 | 0.018 (0.772)    | 0.204 (0.309)    | **0.830 (0.004)**| **0.608 (0.039)**| 0.449 (0.100)    | 0.371 (0.147)    |
| Afinatib                  | 0.000 (0.965)    | 0.188 (0.331)    | **0.599 (0.041)**| **0.672 (0.024)**| 0.386 (0.137)    | 0.248 (0.256)    |
| Gemcitabine               | 0.197 (0.318)    | 0.087 (0.522)    | 0.021 (0.785)    | 0.005 (0.882)     | 0.006 (0.865)    | 0.026 (0.732)    |
| Dasatinib                 | 0.231 (0.275)    | 0.034 (0.691)    | 0.105 (0.478)    | 0.007 (0.854)     | 0.268 (0.234)    | 0.020 (0.763)    |
| Linsitinib                | 0.114 (0.459)    | 0.133 (0.421)    | 0.062 (0.589)    | 0.088 (0.519)     | 0.038 (0.674)    | 0.000 (0.972)    |
| Stattic                   | 0.232 (0.274)    | 0.008 (0.845)    | 0.483 (0.083)    | 0.412 (0.120)     | **0.809 (0.006)**| **0.682 (0.022)**|
| Crenolanib                | 0.034 (0.694)    | 0.236 (0.269)    | 0.010 (0.828)    | 0.189 (0.329)     | 0.232 (0.274)    | 0.000 (0.983)    |
| Bosutinib                 | 0.003 (0.903)    | 0.020 (0.763)    | 0.355 (0.158)    | 0.074 (0.555)     | **0.747 (0.012)**| 0.397 (0.129)    |
| AZD4547                   | 0.001 (0.959)    | **0.751 (0.012)**| 0.063 (0.587)    | 0.053 (0.620)     | 0.054 (0.616)    | 0.040 (0.667)    |
| Ponatinib                 | 0.261 (0.241)    | 0.041 (0.663)    | 0.186 (0.334)    | 0.091 (0.512)     | 0.066 (0.577)    | 0.037 (0.681)    |

**Note:** SPSS software was used to determine the significance and R² value using linear regression analysis where the expression of cell surface marker was independent variable and IC₅₀ value of the drug was the dependent variable. A R² value closer to 1 indicated reliability of the data whereas P-value of <0.05 was considered statistically significant (printed in bold). TKIs, tyrosine kinase inhibitors; CDK, cyclin-dependent kinase; STAT3, signal transducer and activator of transcription 3; IC₅₀, half maximal inhibitory concentration; EGFR, epidermal growth factor receptor; HER, human epidermal growth factor receptor; IGF-IR, insulin-like growth factor 1 receptor; ALK7, anaplastic lymphoma kinase 7.

Figure 5. Continued.
STAT3 signaling in non-small cell lung cancer (NSCLC) was induced by the activation of MET (41). In addition, a significant association was found between c-MET expression and Src/Abl inhibitor bosutinib. The SRC and Abl are some of the intracellular effector molecules recruited following auto-phosphorylation of docking site of activated c-MET (40). Overexpression and activation of the c-MET receptor have been shown to be involved in SRC kinase activation (42).

Cell cycle distribution analysis revealed that the treatment of HPCCLs with afatinib, dasatinib, dinaciclib, stattic and gemcitabine increased the population of cells in sub-G1 with concomitant decrease in the G1 phase. Afatinib treatment also increased cells in the S and G2/M phase in most of the cell lines and similar results were reported following the treatment of nasopharyngeal carcinomas with afatinib (43). Dinaciclib treatment caused S-phase arrest in most of the cell lines which is consistent with the blockage of CDK2, one of the targets of dinaciclib (44). Treatment with dasatinib arrested cells in the S and G2/M phase which is consistent with a study where increasing the dasatinib concentration from 0.5-1.0 µM increased the percentage of BxPC-3 cells in the S-phase (21). Treatment with the cytotoxic agent gemcitabine increased cells in S-phase consistent with the inhibition of DNA replication (45). Treatment with afatinib, dasatinib and stattic inhibited the phosphorylation of EGFR, SRC and STAT3, respectively and the migration of four human pancreatic cancer cell lines, established from patients with either a primary tumor or a metastatic tumor. Our results suggest that treatment with these agents result in inhibition of two hallmarks of cancer which are tumour cell proliferation and migration.

Next, we investigated the combinational potential of most effective drugs on four HPCCLs. We found that treatment with a combination of SRC targeting TKI dasatinib with the pan-HER TKI afatinib resulted in synergistic growth
inhibition of all four HPCCLs examined. Treatment with the combination of dasatinib with gemcitabine also resulted in synergistic growth inhibition of four HPCCLs, including those established from patients with a primary pancreatic tumor (e.g., Bx-PC-3) or liver metastasis (e.g., Capan-1). Consistent with the results of a present study, the latter combination has been reported to have synergistic effect in two other HPCCLs (46,47). Although, dasatinib and gemcitabine combination has been shown to promote stable disease and to induce a partial response in patients with pancreatic cancer (48), it failed to improve patient survival in a phase II trial setting due to increased toxicity of such a combination (49). This combination is currently being used in a phase II trial in pancreatic cancer patients (ClinicalTrials.gov Identifier: NCT01234935). The triple combination of SRC inhibitor (dasatinib), EGFR inhibitor (erlotinib) and gemcitabine demonstrated a synergistic antitumor effect in pancreatic ductal adenocarcinoma (PDAC) as well as encouraging preliminary activity in patients with advanced pancreatic cancer (50,51). However, when afatinib was used in combination with the CDK1/2/5/9 inhibitor, dinaciclib, such a combination had agonistic effect in all cell lines examined. This highlights the importance of selecting the appropriate partner when such drugs are used in combination.

In conclusion, we demonstrated that of various targeting agents employed in our study, the CDK inhibitor dinaciclib, the irreversible pan-HER TKI afatinib, and the SRC targeting TKI dasatinib were most effective at inhibiting the proliferation and migration of HPCCLs, established from both a primary pancreatic cancer and from a metastatic pancreatic cancer. The combination of dasatinib with afatinib and dasatinib with gemcitabine led to synergistic growth inhibition in pancreatic cancer cell lines. Our results support the need for further investigation of the therapeutic potential of these combinations in the treatment of pancreatic cancer.

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Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

TK performed the experiments and conducted data analysis as part of her PhD project. HM conceived the original research idea, read, and revised the manuscript. TK and HM wrote the manuscript. SK and NI carried out data analysis. AGD and SM were the other PhD supervisors on this study and thus were also involved in all aspects of the experiments, data analysis and writing. All authors have read and approved the study.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68: 394-424, 2018.
2. Rawla P, Sunkara T and Gaduputi V: Epidemiology of pancreatic cancer: Global trends, etiology and risk factors. World J Oncol 10: 27-29, 2017.
3. Siegel RL, Miller KD and Jemal A: Cancer statistics, 2020. CA Cancer J Clin 70: 7-30, 2020.
4. Carreras-Torres R, Johansson M, Gaborieau V, Haycock PC, Wade KH, Relton CL, Martin RM, Davey Smith G and Brennan P: The role of obesity, type 2 diabetes, and metabolic factors in pancreatic cancer: A mendelian randomization study. J Natl Cancer Inst 109: djx012, 2017.
5. Lauby-Secretan B, Scoccianti C, Loomis D, Grosse Y, Bianchini F and Straif K; International Agency for Research on Cancer Handbook Working Group: Body Fatness and Cancer-Viewpoint of the IARC Working Group, N Engl J Med 375: 794-798, 2016.
6. Wolfgang CL, Herman JM, Laheru DA, Klein AP, Erdek MA, Fishman EK and Hruban RH: Recent progress in pancreatic cancer. CA Cancer J Clin 65: 318-348, 2015.
7. Ioannou N, Sędziow AM, Dalgleish A, Mackintosh D and Modjtabahedi H: Expression pattern and targeting of HER family members and IGF-IR in pancreatic cancer. Front Biosci (Landmark Ed) 2012. 17: 2698-2724, 2012.
8. Seshacharyulu P, Ponnamsetty MP, Haridas D, Jain M, Ganti AK and Butra SK: Targeting the EGFR signaling pathway in cancer therapy. Expert Opin Ther Targets 16: 15-31, 2012.
9. Tebbutt N, Pedersen MW and Johns TG: Targeting the ESRB family in cancer: Couples therapy. Nat Rev Cancer 13: 663-673, 2013.
10. Li Q, Zhang L, Li X, Yan H, Yang L, Li Y, Li T, Wang J and Cao B: The prognostic significance of human epidermal growth factor receptor family member protein expression in operable pancreatic cancer: HER1-4 protein expression and prognosis in pancreatic cancer. BMC Cancer 16: 910, 2016.
11. Perini MV, Montagnini AL, Coudry R, Patzina R, Penteado S, Abdo EE, Diniz A, Jukemura J and da Cunha JE: Prognostic significance of epidermal growth factor receptor overexpression in pancreas cancer and nodal metastasis. ANZ J Surg 85: 174-178, 2015.
12. Ahn JH, Choi KH, Lee JH and Lee HS: The predictive value of EGFR expression in pancreatic ductal adenocarcinoma. J Cancer Res Clin Oncol 138: 83-90, 2012.
13. Jin J, Wang L, Sun Q, Liu D, Yu X, Li L and Wang Y: The association of EGFR expression with clinical outcome in pancreatic cancer patients. J Cancer Res Clin Oncol 138: 77-82, 2012.
14. Moore MJ, Goldstein D, Hammond M, Carpenter JR, Gallinger S, Au HJ, Murawala P, Walde D, Wolff RA and et al: Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: A phase III trial of the National Cancer Institute of Canada clinical trials group. J Clin Oncol 25: 1960-1966, 2007.
15. Ioannou N, Seddon AM, Dalgleish A, Mackintosh D and Modjtahedi H: Treatment with a combination of the ErbB (HER) family blocker afatinib and the IGF-IR inhibitor, NVP-AEW541 induces synergistic growth inhibition of human pancreatic cancer cells. BMC Cancer 13: e14, 2013.

16. Nones K, Waddell N, Song S, Patch AM, Miller D, Johns A, Wu J, Kassahn KS, Wood D, Bailey P, et al.: Genome-wide DNA methylation patterns in pancreatic ductal adenocarcinoma reveal epigenetic deregulation of SLIT-ROBO, JTG2A, and MET signaling. J Clin Oncol 30: 1110-1118, 2012.

17. Zhou W, Jubb AM, Lyle K, Xiao Q, Ong CC, Desai R, Fu L, Gnaf F, Song Q, Haverty PM, et al.: PAK1 mediates pancreatic cancer cell migration and resistance to MET inhibition. J Pathol 234: 502-513, 2014.

18. Zhou WH, Huang XQ, Ou ZJ, Liu J, Zhang ZH, Zhao N, Feng ZZ and Lv XH: Expression and prognostic significance of CD151, c-Met, and integrin alpha3/alpha6 in pancreatic ductal adenocarcinoma. Dig Dis Sci 56: 1090-1098, 2011.

19. Liu C, Yang Z, Li D, Liu Z, Miao X, Yang L, Zou Q and Yuan Y: Overexpression of B2M and loss of ALK7 expression are associated with invasion, metastasis, and poor-prognosis of the pancreatic ductal adenocarcinoma. Cancer Biomark 15: 735-743, 2015.

20. Shields DJ, Murphy EA, Desrosserles JS, Mielpo A, Lau SK, Barnes LA, Lespérance J, Huang M, Schmidt C, Tarin D, et al.: Oncogenic Src family kinase cooperativity in pancreatic neoplasia. Oncogene 30: 2123-2134, 2011.

21. Nagaraj NS, Smith JJ, Revetta F, Washington MK and Merchant NB: Targeted inhibition of SRC kinase signaling attenuates pancreatic tumorigenesis. Mol Cancer Ther 9: 2322-2332, 2010.

22. Morton JP, Karim SA, Graham K, Tipnis S, Jamieson N, Merchant NB: Dasatinib inhibits the development of metastases in a mouse model of pancreatic ductal adenocarcinoma. Gastroenterology 139: 903-913, 2010.

23. Chee CE, Krishnamurthi S, Nock CJ, Meropol NJ, Gibbons J, Fu P, Bokar J, Teston L, O’Brien T, Gudena V, et al.: Phase II study of dasatinib (BMS-354825) in patients with metastatic adenocarcinoma of the pancreas. Oncologist 18: 1091-1092, 2013.

24. Cross J, Raffenne J, Couvelard A and Poté N: Tumor heterogeneity in pancreatic adenocarcinoma. Pathobiology 85: 64-71, 2018.

25. Yao W, Maitra A and Ying H: Recent insights into the biology of pancreatic cancer. Curr Opin Genet Dev 33: 257-264, 2016.

26. Haeberle L, Desrosier JS, Mielpo A, Lau SK, Barnes LA, Lespérance J, Huang M, Schmidt C, Tarin D, et al.: Oncogenic Src family kinase cooperativity in pancreatic neoplasia. Oncogene 30: 2123-2134, 2011.

27. Ioannou N, Kassahn KS, Wood D, Bailey P, et al.: Genome-wide DNA methylation patterns in pancreatic ductal adenocarcinoma reveal epigenetic deregulation of SLIT-ROBO, JTG2A and MET signaling. J Clin Oncol 30: 1110-1118, 2012.

28. Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM and Matrisian LM: Projecting cancer incidence and deaths to 2030: The unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer Epidemiol Biomark Prev 22: 2933-2941, 2013.

29. Adel N: Current treatment landscape and emerging therapies for pancreatic cancer. Am J Manag Care 25 (1 Suppl): S3-S10, 2019.

30. Paruch K, Dwyer MP, Doll R, Wiswell D, Seghezzi W, Paruch K, Dwyer MP, et al.: Analysis of clinical features and survival of patients with comorbid conditions associated with advanced solid tumors. Invest New Drugs 31: 918-926, 2013.

31. Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM and Matrisian LM: Projecting cancer incidence and deaths to 2030: The unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer Epidemiol Biomark Prev 22: 2933-2941, 2013.

32. Al-U’datt DGF, Al-Husein BAA and Qasaimeh GR: A mini-review of c-Met as a potential therapeutic target in malignancies. Int J Clin Exp Pathol 8: 194-202, 2015.

33. Adel N: Current treatment landscape and emerging therapies for pancreatic cancer. Am J Manag Care 25 (1 Suppl): S3-S10, 2019.

34. Athineos D, Doyle B, McKay C, Heung MY, Oien KA, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM and Matrisian LM: Projecting cancer incidence and deaths to 2030: The unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer Epidemiol Biomark Prev 22: 2933-2941, 2013.

35. Nagaraj NS, Washington MK and Merchant NB: Combined blockade of Src kinase and epidermal growth factor receptor with gemcitabine overcomes STAT3-mediated resistance of inhibition of pancreatic tumor growth. Clin Cancer Res 17: 483-493, 2011.