**Therapeutic developments in pancreatic cancer: breaking through**

John P. Neoptolemos1, Jörg Kleeff2, Patrick Michl3, Eithne Costello1, William Greenhalf1, Dan Palmer1

**Affiliation**

1Department of Molecular and Clinical Cancer Medicine, Institute of Translational Medicine, University of Liverpool, L69 3GA, UK

2Department of Visceral, Vascular and Endocrine Surgery, Martin-Luther-University Halle-Wittenberg, 06120 Halle (Saale), Germany

3Department of Visceral, Vascular and Endocrine Surgery, Martin-Luther-University Halle-Wittenberg, 06120 Halle (Saale), Germany

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**Abstract**

The overall 5-year survival rate for pancreatic cancer has changed little for some time and pancreatic cancer is predicted to be the second leading cause of cancer-related mortality in the next decade. Yet there are improvements in first and second line palliative therapies and significant improvements in survival rates with adjuvant treatment. The use of biomarkers to help define treatment and the potential of neoadjuvant treatments also offer opportunities. This article brings together information on what has been achieved to date, what is working currently and where successes are likely to be had in the future. It addresses the questions of how we should approach developing treatments for pancreatic cancer. This includes patients with metastatic, locally advanced and borderline resectable pancreatic cancer, as well as patients with resected pancreatic cancers. In addition to embracing newer strategies comprising genomics, stromal- and immunotherapies, conventional approaches still offer considerable prospects for greater traction and synergy with evolving concepts.

**Key words:** pancreatic cancer, adjuvant therapy, neoadjuvant therapy, targeted therapy, immunotherapy, local ablative therapy

**Introduction**

Pancreatic cancer therapy remains a formidable challenge. In part due to improvements in the treatment of other cancers and an aging population, pancreatic cancer will likely become the second leading cause of cancer-associated mortality within the next decade 1. Its worldwide incidence is predicted to be around 420,000 by the year 2020 with an associated mortality of around 410,000 2. Our ever-growing understanding of the complex genetic, epigenetic, and metabolic alterations as well as of the equally complex interplay of cancer cells with stromal cells, immune cells, and endothelial cells has not yet resulted in a dramatic change in the overall outcome for pancreatic cancer patients 3. Challenges include identification of populations at risk for screening and prevention, early detection by advanced imaging and novel cancer (bio)markers, and most notably better therapeutic options that overcome the resistance of pancreatic cancer to current treatment modalities including chemotherapy, radiotherapy and targeted therapies. Here, we provide a perspective on current and future pancreatic cancer therapy.

**Surgical resection and conventional chemotherapy for resectable and borderline resectable pancreatic cancer pancreatic cancer: a step change.**

Surgery remains the only chance for cure of pancreatic cancer and it has evolved from a high-risk procedure a few decades ago to a challenging, yet relatively safe procedure in experienced centers 3,4. Surgery alone, however, is not enough, as more than 90% of patients relapse and die of their disease after potentially curative surgery without additional therapy. Adjuvant treatment strategies have therefore been evaluated during the last several decades (table 1).

Historically, the Gastrointestinal Tumor Study Group (GITSG) compared observation following surgery to adjuvant 5-fluorouracil (5-FU)-based radiation, followed by weekly 5-FU for 2 years or until recurrence in 43 patients and demonstrated a significant difference in favor of the treatment arm 5. Although this trial was carried out in the 1970s, it has influenced patient care until today, especially in the US. In the 1980s to 1990s the EORTC trial compared adjuvant radiotherapy and 5-FU versus observation. No significant survival benefit for this treatment, either in the pancreatic head cancer subgroup or in the whole cohort, was found 6.

The ESPAC-1 trial randomized 541 patients into a two-by-two factorial design (70 5-FU based chemoradiotherapy, 74 5-FU chemotherapy, 72 both, 69 observation) and a further 68 patients to chemoradiotherapy or no chemoradiotherapy and 188 to chemotherapy or no chemotherapy, and showed no benefit for chemoradiation but a potential benefit for chemotherapy 7. Follow-up analysis of the patients randomized within the two-by-two factorial design 8 demonstrated a significant benefit of adjuvant chemotherapy (5-FU) following R0/R1 resection of pancreatic cancer. The CONKO-001 trial similarly showed a clear benefit of adjuvant gemcitabine versus observation 9,10. Subsequently the ESPAC-3 trial compared 5-FU versus gemcitabine as adjuvant therapy and showed no improved survival for gemcitabine compared to 5-FU, although treatment-related serious adverse events were significantly higher in the 5-FU group 11. Importantly, completion of all 6 cycles of chemotherapy rather than early initiation was an important prognostic factor 12. The addition of erlotinib to gemcitabine did not improve survival in R0 resected pancreatic cancer patients 13. In a trial of R0 resected pancreatic cancer patients in Japan (69% with a performance status of 0), S-1 was superior to gemcitabine as adjuvant therapy, with more than 40% 5-year survival rates for the S-1 group 14. This finding remains to be confirmed in non-Asian populations.

The most recent ESPAC-4 trial compared gemcitabine to gemcitabine/capecitabine combination therapy in R0/R1 resected pancreatic cancer patients 15. This trial demonstrated the superiority of gemcitabine/capecitabine in the adjuvant setting with 5-year survival rates approaching 30%, even though approximately 80% of the tumors were N1 and 60% R1 (<1mm of the margin), and only 42% had a performance status of 0. There was slightly more toxicity in the gemcitabine/capecitabine arm but overall this was manageable and not significant 15. The ESPAC-4 trial now sets the standard and benchmark for adjuvant combination cytotoxic therapy, pending results of the nab-paclitaxel plus gemcitabine (APACT) and mFOLFIRINOX (PRODIGE) trials in this setting.

An emerging strategy for pancreatic cancer, especially for borderline resectable 16 (but also for resectable as well as locally advanced unresectable cancers) is neoadjuvant/perioperative therapy 17. For many decades, high quality data from randomized controlled trials in the neoadjuvant setting are lacking, partly because of difficulties in trial design and recruiting patients. Indeed, the first randomized trial for neoadjuvant chemoradiotherapy in pancreatic cancer had to be closed early for the latter reason and results were not significant 18. Currently several randomized phase III trials are underway that will hopefully provide data on this concept. In resectable patients for example, the NEOPAC study analyses adjuvant gemcitabine versus neoadjuvant gemcitabine/oxaliplatin plus adjuvant gemcitabine 19 while the NEOPA trial tests neoadjuvant chemoradiotherapy versus upfront surgery 20. Similarly, the PREOPANC trial analyses the same concept for resectable as well as borderline resectable pancreatic cancers 21. The ESPAC-5f trial compares, in a four arm design, immediate surgery, neoadjuvant chemoradiotherapy, gemcitabine/capecitabine and FOLFIRINOX in borderline resectable pancreatic cancer patients 22.

With more active chemotherapeutic regimens available, most notably FOLFIRINOX, there has been a shift away from radiochemotherapy towards chemotherapy in the neoadjuvant setting. A number of centers have reported high resection rates even in locally advanced, unresectable cases following neoadjuvant FOLFIRINOX therapy 23-25 (up to 60% in one report; although 50% of the cases were initially unresectable because of distant metastasis 26). Importantly, response to therapy is not reflected by imaging 27, highlighting the need for multidisciplinary discussions and, on a case-by-case decision, surgical exploration.

There is hope that with more active and evidence-based treatment options becoming available, a larger proportion of patients with borderline resectable or locally advanced unresectable tumours 16 will be resected, significantly increasing the overall number of patients that can be offered the only chance for cure.

**Local therapies for locally advanced pancreatic cancer: new methods.**

A sizable proportion of pancreatic cancer patients, estimated at around 30-40%, present with borderline resectable or locally advanced unresectable tumors 3. While borderline resectable tumors are either candidates for upfront surgery or neoadjuvant treatment protocols (see above), treatment for locally advanced tumors is more complex and less evidence based. There is the general agreement that these tumors should initially be treated with systemic (induction) chemotherapy, the argument being that a relevant number of these tumors has already metastasized and that local therapies would be of questionable benefit in those cases. If the tumors do not show metastatic progression after initial systemic therapy, local therapies are considered to be an option for tumor control and/or symptom relief. These methods include irreversible electroporation (IRE), radiofrequency ablation (RFA), stereotactic body radiation (SBRT), high-intensity focused ultrasound (HIFU), and others (reviewed in 28-30) (table 2). RFA and SBRT are the best studied modalities in locally advanced pancreatic cancer. RFA is usually carried out during open surgery or EUS guided and mortality is reported to be 0-3% with relevant morbidity in the range of 4-28%. SBRT has been studied with varying techniques and radiation doses applied and morbidity has been reported in up to 25%. IRE is a method that has gained interest since it is thought to be able to destroy tumor tissue in the vicinity of critical structures, i.e. vasculature, that other methods relying on thermal effects like RFA cannot. Morbidity and mortality rates are within the range of what has been reported for RFA and SBR; importantly IRE can also be carried out percutaneously under CT guidance. HIFU has been less well studied 31,32 as have been the other potential local options 28-30.

Studies analyzing local ablative therapies have reported tumor regression, prolonged survival symptom control as well as tumor resection in a number of patients. However, data regarding effectivity are heavily biased in the published patient cohorts and thus conclusive data regarding the effects of local ablative therapies in pancreatic cancer are sparse owing to the lack of randomized controlled trials. Nonetheless, as more solid data become available these modalities might offer new options in the multimodal therapy of locally advanced pancreatic cancer patients.

**Conventional chemotherapy for advanced pancreatic cancer: incremental progress.**

Patients with distant metastases and/or local irresectability generally qualify for systemic palliative chemotherapy. Until 2011, monotherapy with gemcitabine remained the standard of care based on a trial by Burris and co-workers who compared gemcitabine monotherapy to 5-FU monotherapy. The study showed a significant clinical benefit but only marginally extended survival in favour of gemcitabine 33. Combinations of gemcitabine with a second cytotoxic drug or various targeted agents have been extensively evaluated in recent years. Most of these trials, however, proved to be futile, leaving gemcitabine as the sole standard of care. The situation changed in 2011, when the PRODIGE 4/ACCORD 11 trial demonstrated a clinically meaningful survival advantage of the gemcitabine-free FOLFIRINOX regimen (folinic acid, fluorouracil, irinotecan and oxaliplatin) over gemcitabine in patients with metastatic pancreatic cancer (11.1 vs. 6.8 months overall survival) 34. In 2013, another combination therapy entered the stage with the phase III MPACT trial reporting the results of nano-formulated albumin-bound paclitaxel (nab-paclitaxel) in combination with gemcitabine in patients with metastatic pancreatic cancer 35. In contrast to conventional paclitaxel requiring a castor oil based solvent which frequently leads to infusion hypersensitivity reactions, nab-Paclitaxel represents a solvent-free, albumin-bound and water-soluble formulation of paclitaxel. This novel formulation significantly reduces the risk of hypersensitivity reactions and neutropenia, with no need for pre-medications with antihistamines and systemic steroids, as required for conventional paclitaxel 36. The MPACT trial revealed a significant survival benefit for nab-paclitaxel combined with gemcitabine over gemcitabine monotherapy (8.7 versus 6.6 months median overall survival) 37.

The patient populations in the PRODIGE 4/ACCORD 11 and the MPACT trials showed distinct differences regarding mean patient age, metastatic sites, CA19-9 levels and ECOG status. This clearly precludes any head-to-head comparison between the trial results. Both available combination regimens for first-line therapy of metastatic pancreatic cancer, FLOFIRINOX and nab-paclitaxel/gemcitabine, are associated with a significant toxicity profile. Neutropenia occurs in approximately 47.5% of FOLFIRINOX-treated patients and in 38% of nab-paclitaxel/gemcitabine-treated patients, with 5.4% and 3% febrile neutropenia, respectively 34,37. Patients must be carefully selected for combination therapies. FOLFIRINOX is mainly reserved for patients with a ECOG 0-1 performance status without limiting comorbidities, nab-paclitaxel/gemcitabine may also be considered in selected patients with ECOG 2 performance status. Of note, both FOLFIRINOX and Nab-paclitaxel/gemcitabine combination therapies have been evaluated in randomized clinical trials (RCT) for patients with metastatic disease only. Large RCTs evaluating both protocols for locally advanced disease with or without the addition of sequential chemoradiation are ongoing. Nevertheless, both intensified combination chemotherapy protocols are frequently used in an attempt to downstage locally advanced tumours, either with chemotherapy alone or with a sequential approach in which induction chemotherapy is followed by chemoradiation or other local therapies as described above in patients without evidence of progression during induction chemotherapy.

**Second line therapy: a new beginning.**

Most pancreatic cancers progress within a relatively short time during or after first line palliative chemotherapy despite of the use of intensified protocols. Thus, second line chemotherapy is important in a relevant proportion of pancreatic cancer patients. Around 16-86% of patients undergo second line therapy 38; in the ACCORD/PRODIGE-4 trial, roughly 50% of patients (165 of 342) underwent second line chemotherapy with a median survival of 4.4 months 34. Despite the unmet need of evidence-based second line therapies, only a few phase III randomized controlled trials have been carried out (table 3). The first phase III randomized controlled trial compared best supportive care to oxaliplatin, 5-fluorouracil and folinic acid (OFF) in 46 patients 39. The trial started in 2002 and had to be stopped in 2003 because of low recruitment. Nonetheless, the OFF regimen was associated with a significant increased second line survival (4.8 vs. 2.3 months; p=0.008). The follow-up trial compared OFF with 5-fluorouracil and folinic acid (FF) and demonstrated that in gemcitabine refractory pancreatic cancer patients, OFF is superior to FF (median survival 5.9 vs. 3.3 months, p=0.01) and that the addition of oxaliplatin did not result in relevantly increased toxicity 40. In contrast, the PANCREOX trial did not show a benefit of the addition of oxaliplatin (FOLFOX6) to 5-fluorouracil and folinic acid (median survival 6.1 vs. 9.9 months, p=0.02) with increased toxicity being observed in the FOLFOX6 arm 41. A recent phase III RCT demonstrated that nanoliposomal irinotecan plus 5-fluorouracil and folinic acid significantly increased survival compared to 5-fluorouracil and folinic acid (6.1 vs. 4.2 months, p=0.012) in patients previously treated with a gemcitabine based regimen 42. Most studied carried out to date have evaluated second line regimens after gemcitabine based therapy failure. FOLFIRINOX is an active and more frequently used regimen in fit patients 34; however, data regarding second line therapy following this regimen are sparse. In a prospective multicentre cohort study, 57 patients were treated with Nab-paclitaxel and gemcitabine following FOLFIRINOX, with a median survival of 8.8 months and an acceptable toxicity profile 43.

There is now evidence from phase II and III trials 38 that second line therapy is effective and well tolerated. Median survival after disease progression with first line chemotherapy has increased from around 2-3 months to 4-5 months and to more than 6 months in recent trials 38,42. Due to the emergence of more active first line therapies, novel second line therapies have to be established and evaluated (table 4). As of now, nanoliposomal irinotecan (or oxaliplatin) and 5FU/FA seems to be the best option in gemcitabine-(based) pretreated patients, whereas a gemcitabine based regimen (e.g. Nab-paclitaxel plus gemcitabine) would be an option after FOLFIRINOX failure.

**Targeted therapies: failures and hope.**

Numerous targeted agents have been evaluated alone or in combination with chemotherapy in metastatic pancreatic cancer. Unfortunately, most agents have so far failed to significantly improve patient survival. The long list of targeted compounds tested in trials as futile include antiangiogenic drugs such as the vascular endothelial growth factor (VEGF) inhibitors bevazicumab and aflibercept 44,45 as well as multikinase inhibitors with antiangiogenic activity such as sunitinib, sorafenib and axitinib 46-49x. It may be speculated that the futility of all antiangiogenetic approaches tested so far is due to the largely hypovascular nature of the stroma surrounding cancer cells in this disease 48. More recently, other compounds targeting important signaling cascades in pancreatic cancer proved futile in randomized trials with gemcitabine as chemotherapeutic backbone, among them the IGF1R antibodies ganitumab and cixutumumab 50,51, the multi-kinase inhibitor masitinib 52 and the PI3K inhibitor rigosertib 53; for review: 54.

The only targeted agent, which demonstrated a clinically marginal, though statistically significant effect on patient survival is erlotinib, a small molecule inhibitor of the EGFR tyrosine kinase. Moore et al. reported in a randomized trial that the combination of gemcitabine and erlotinib conferred a mean survival benefit of approximately two weeks over gemcitabine alone 55. This marginal benefit clearly raises questions about the clinical significance of erlotinib. It may be speculated that due to the high percentage of activating K-Ras mutations occurring in up to 90% of pancreatic cancer patients, pharmacological inhibition of EGFR upstream of K-Ras remains only minimally effective in this cancer type 48. Notably, a subgroup of patients that develop a skin rash (grade ≥ 2) upon erlotinib showed a median survival of almost 12 months. While the underlying molecular mechanisms behind this striking observation remain to be fully elucidated, skin rash can be generally considered as a positive predictive marker for response to anti-EGFR therapy across tumor entities 56.

The failure of targeted therapies to improve the outcome of advanced pancreatic cancer in unselected patient populations may be explained by both the high molecular heterogeneity of this disease and the high content of surrounding stromal and inflammatory components impacting on signaling pathways, drug accessibility, half-life and metabolism of the drugs in the tumors. In addition to addressing the crosstalk with mesenchymal and inflammatory stromal components (see below) mediating therapy resistance, advances in determining the key molecular drivers of each tumor in a precision medicine approach will hopefully overcome the disappointing performance of targeted approaches observed in unselected trail populations of advanced pancreatic cancer.

**Stroma targeting: learning the hard way.**

The pancreatic cancer tumour microenvironment has attracted much interest in recent years, with particular attention focused on its role as a determinant of therapy response. The plentiful fibrotic stroma associated with pancreatic cancer contains a variety of cell types, including cancer-associated fibroblasts (CAFs), inflammatory cells, blood vessels, and nerve cells. The stroma also contains a variety of extracellular matrix components, with pancreatic CAFs activated to produce collagen, fibronectin, laminin and hyaluronic acid (figure 1).

Exploiting the stoma in order to enhance chemotherapeutic drug delivery is an attractive prospect. Secreted protein acidic and rich in sustaining (SPARC) is a matricellular protein produced by CAFs that is known to bind albumin. Consequently, it was proposed that SPARC might enrich the concentration of the albumin bound formulation of paclitaxel (Nab-paclitaxel) within the pancreatic cancer tumour microenvironment, thereby enhancing its anti-tumour activity. As mentioned above, the combination of gemcitabine plus Nab-paclitaxel led to improved survival compared to gemcitabine monotherapy (8.5 months versus 6.7 months, p<0.001) 37. However, no association was established between stromal SPARC levels and overall survival in either treatment arm 57.

The deposition of extracellular matrix components alters the physical nature of the developing pancreatic tumour, causing stiffness and increasing hydrostatic pressures. Furthermore, pancreatic tumours are poorly vascularised. The combination of increased hydrostatic pressure within pancreatic tumours and poor vascularisation is believed to create a barrier to drug uptake and several approaches aimed at challenging this stromal barrier to enhance drug delivery to PDAC tumour cells have been tested. Systemic administration of a modified hyaluronidase molecule, PEGPH20 into mice with established pancreatic tumours decreased tumoral hyaluronic content, reduced interstitial fluid pressures and increased the number of functioning tumour blood vessels 58,59. This promising preclinical data led to clinical trials in which PEGPH20 is combined with nab-paclitaxel and gemcitabine or with FOLFIRINOX (ClinicalTrials.gov number, NCT01839487 and NCT01959139 respectively). Interim analysis of the nab-paclitaxel and gemcitabine trial showed that patients with high levels of tumoural hyaluronic acid had progression free survival of 9.2 months when treated with the combination that included PEGPH20 compared to 4.3 months if given paclitaxel and gemcitabine only 60.

Pancreatic stellate cells (PSCs) are a form of fibroblast that become activated in the developing pancreatic tumour. Reversing activated PSCs to a quiescent phenotype may decrease fibrosis and improved drug delivery. PSCs express abundant vitamin D receptor. Treatment with calcipotriol, a ligand for this receptor, was associated with the reversion of activated PSCs to a quiescent state and in a preclinical model, led to decreased fibrosis, and enhanced the uptake of gemcitabine to tumours 61.

However, targeting the fibroblastic components of PDAC needs to be approached with caution. Hedgehog signalling between cancer cells and CAFs promotes stromal desmoplasia. Short-term pharmacological inhibition of the sonic hedgehog (SHH) pathway using Smo inhibitor IPI-926 inhibited myofibroblast growth and collagen deposition, increased tumour vascularity and enhanced gemcitabine delivery to pancreatic tumours in a mouse model 62. However, the use of combined IPI-926 and gemcitabine in patients failed to confirm improved survival over gemcitabine monotherapy. It has since been demonstrated that longer term IPI-926 administration decreased stroma and increased vascularisation of pancreatic tumours in a preclinical mouse model, but such tumours surprisingly showed accelerated tumour growth and increased metastasis 63. Likewise, genetically eliminating stromal fibroblasts caused an aggressive tumour phenotype and decreased survival 64. Thus, the stroma appears to protect against tumour progression, while at the same time creates an environment that impairs drug delivery to the tumour. More research is required to understand how to target the stroma optimally so as not to compromise its protective role.

The hypoxic nature of the pancreatic tumour microenvironment offers the possibility of selectively targeting cancer cells. Evofosfamide is a prodrug that, under hypoxic conditions, releases the DNA alkylating agent, bromo isophosphoramide mustard (Br-IPM). Br-IPM inhibits DNA replication by forming DNA crosslinks, and interferes with DNA transcription. In the phase III clinical trial MAESTRO, 693 patients with locally advanced unresectable or metastatic pancreatic cancer were randomised to combined evofosfamide and gemcitabine versus combined placebo and gemcitabine (NCT01746979). Despite a significant improvement in median progression free survival from 3.7 months with the placebo-containing combination to 5.5 months with the evofosfamide-containing combination; HR = 0.77 (95% CI: 0.65–0.92, p = 0.004), a slight improvement in median overall survival for patients who received evofosfamide/gemcitabine (8.7 months) compared to patients who received placebo/gemcitabine (7.6 months) did not reach statistical significance; HR = 0.84 (95% CI: 0.71–1.01, p = 0.059) 65.

**Immunotherapies: great promise.**

A key reason why cancer cells can survive and establish tumours is that they can suppress the immune response, either directly or via other cells in the tumour microenvironment. Indeed this has now been defined as one of the hallmarks of cancer 66. Immune response also predicts survival showing that if this can be manipulated there will be a clinical benefit 67.

Mechanisms by which cancer suppresses the immune response include activation of regulatory T-cells (T-Regs) 68 or myeloid derived suppressor cells (MDSCs)69; inhibition of effector T-cells (T-Effs) 70 or antigen presenting cells (APCs) 71 and modulation of macrophage populations within the tumour 72. It also includes establishing a barrier to entry of T-Effs into the tumour milieu 73. Immunotherapy to reverse these tumour associated effects has been successful in many tumour types: a notable exception to date being pancreatic cancer 74.

Figure 2 shows various forms of immunotherapy. The simplest form is a cancer vaccine using a tumour specific antigen 75. Unfortunately, such direct approaches have as yet proved ineffective in pancreatic cancer 74, the Telovac trial for example gave no survival benefit in patients with advanced pancreatic cancer 76. Other trials such as the Ras peptide vaccine TG01/GM-CSF are ongoing 77.

Adoptive approaches involve injecting immune cells into patients. This could be T-cells isolated from the patient: T-Effs that have infiltrated a patient’s tumour (Tumour infiltrating lymphocytes, TILs) have already shown responsiveness to tumour antigens and an ability to migrate from the vasculature into the tumour microenvironment. Therefore, expanding TILs ex-vivo and injecting them back into the patient could be beneficial 78. Similarly, extracting antigen presenting cells (e.g. dendritic cells) and challenging them ex-vivo with an appropriate antigen could provide an agent to stimulate an anti-tumour immune response, for example Sipuleucel in prostate cancer 79. A cheaper and arguably more effective approach is to generate a cell line that will express HLA-DR and, because the cell line is not autologous, proteins that will stimulate an immune response, such as hTert. Phase II clinical trials are underway with one such agent (ACIT-1) in pancreatic cancer. Alternatively, immune cells could be engineered so as to replace the body’s compromised lymphocytes. Use of TCR engineered T-cells showed promise in the laboratory 80 but the technology has largely proved ineffectual or worse in clinical trials 81. In this context, Chimeric Antigen Receptor (CAR) T-cells have shown greater promise 82. These are T-Effs engineered in vitro to express a protein binding domain on their surface, typically in the form of an ScFv, which is grafted onto T-cell stimulatory domains (e.g. third generation CAR T-cells may have a combination of CD3ζ, CD28 and OX40 domains) 83. A cancer specific surface protein is selected as a target producing a therapeutic that can be both specific and effective particularly with lymphodepletion to remove T-Regs and reduce competition for stimulatory cytokines. Further advances, including engineering CAR-T cells to produce appropriate cytokines or suicide cassettes to limit toxicity, are in the pipeline 84. However, CAR T-cells are subject to the same cancer directed inhibition as indigenous T-Effs, which limits their effectiveness.

Cancer activates inhibitors of T-Effs including increasing the number of MDSCs via activation of MDSC CXCR2 with IL-8. MDSCs can be directly targeted with agents interfering with arginase 1 and nitric oxide synthase expression such as the Phosphodiesterase-5 inhibitor Tadalafil 85,86. MDSCs will activate PD-1 via its ligand (PD-L1, B7-H1) 87. PD-L1 is also expressed on the surface of the cancer cells themselves 88 and on M2 macrophages 89. Antagonists of PD-1 (e.g. Nivolumab) or PD-L1 (e.g. Ipilimumab) at least partly counteract cancer induced immune suppression 79. The CD28 homologue CTLA4 (CD152) is expressed on both T-Eff and T-Reg cells and negatively regulates activation, possibly via competitive inhibition of CD2890. In addition, indoleamine 2,3-dioxygenase (IDO) expressed in APCs and MDSCs is activated by CTLA4 via CD80/86 increasing the level of Kynurenine and decreasing tryptophan 91. Depletion of tryptophan is directly immunosuppressive in APCs and kynurenine also acts to inhibit T-Effs via aryl hydrocarbon receptors 91,92. CTLA4 can be directly inhibited with agents such as Ipilimumab 93 and IDO can be inhibited with compounds such as Indoximod 94. As an alternative to inhibiting immunosuppressors, agonists of stimulatory proteins on T-Effs or APCs have potential to restore immune response. MOXR0916 stimulates OX40 on T-Effs 95, CP-870,893 stimulates CD40 on antigen presenting cells96. However, to date none of these antagonists or agonists have proved successful in treatment of pancreatic cancer.

Another way that cancer protects itself is to promote the differentiation of macrophages into cells that promote growth and repair (M2 type macrophages) rather than growth inhibitory killer macrophages (M1-type). M2 macrophages can suppress T-Effs by secreting IL-10 and by producing carbon monoxide via the enzyme Haem Oxygenase (HO); HO can be chemically inhibited with Imidazole-dioxolanes. Innate immunity can also be improved by increasing the proportion of M1 macrophages (Macrophage Innate Conversion, MIC) 72. Toll like receptor 7 (TLR7) on macrophages and MDSCs will promote differentiation into M1 macrophages and the receptor can be stimulated by agents such as Imiquimod 97. However, TLR7 is present on numerous cell types and the result of treatment with an agonist is consequentially complex and ironically inhibition rather than activation of the receptor has been suggested for prevention and treatment of pancreatic cancer 98.

The failure of immunotherapy in treating pancreatic cancer may reflect the efficient exclusion of T-Effs in this form of cancer. This may relate to a subclass of cancer associated fibroblasts which express Fibroblast Activation Protein (FAP). FAP is expressed on fibroblasts and on some macrophages. Depletion of macrophage or fibroblast FAP expressing cells restores immune control in mouse models, suggesting they both have a role in immunosuppression 99. FAP positive fibroblasts express CXCL12 which can form a dimer with HMGB1 (expressed by pancreatic cancer cells) to activate the suppressor CXCR4 on T-Effs. In mouse models, inhibition of CXCR4 using AMD3100 (Plerixafor) allowed accumulation of T-Effs in the tumour 73.

Checkpoint inhibitors and other forms of immunotherapy do promise much for the future treatment of pancreatic cancer, but as seen in Figure 2 millions of years of evolution have led to an extremely complex regulatory network to ensure that the immune response is measured. Therapeutic intervention to restore immune response is likely to lead to beneficial as well as adverse consequences, including diabetes and pancreatitis 100. Balancing the therapy will be difficult and will probably require multiple agents, trials of combinations are underway and the results will need to be carefully monitored 101.

**Precision medicine: opportunities and obstacles.**

Precision medicine is an emerging concept in oncology offering improved outcome by individualizing patient therapy. The key point is to select the best therapy or combination therapy for the right patient 102. This would not only take into account targetable alteration of a specific tumor, but also drug delivery, drug metabolism and side effects for a specific patient. There are a number of obstacles in implementing/developing precision medicine for pancreatic cancer. The incidence for targetable alteration varies and is in general considerably lower than the tumor incidence highlighting the need for validated and accurate biomarkers for enrichment of eligible patients. Further, there is the need to base individual therapies on evidence, necessitating a shift in current preclinical analysis 103 as well as trial designs and evidence generation (reviewed in 102). These trial designs include basket trials that test drugs in different cancers that share common alterations and umbrella trials that test different drugs targeting different alterations in a single tumor type. A first umbrella trial on precision medicine in pancreatic cancer -the Individualized Molecular Pancreatic Cancer Therapy (IMPaCT) trial 104- highlighted the difficulties of this approach. The aim of the trial was to assess standard chemotherapy (gemcitabine) versus personalized therapy. Patients with mutations in homologous recombination and DNA damage repair genes (BRCA1, BRCA2, PALB2 or ATM), with amplified HER2 or with absence of mutations in KRAS would receive targeted treatment. Of 93 patients considered, 76 were screened and 22 eligible patients were identified. However, for various reasons, none of these patients has been successfully treated within the IMPaCT study 104.

There are several individualized treatment options available for pancreatic cancer patients, although most of them are not based on data from controlled clinical trials. For example, patients with evidence of systemic inflammation (as determined by elevated CRP) seem to benefit from combination therapy of the JAK1/JAK2 inhibitor Ruxolitinib with Capecitabine 105. Patients with high ACOX1 expression might benefit from therapy of tyrosine kinase inhibitor Masitinib with gemcitabine 52. Similarly, patients with high levels of hyaluronic acid (HA) respond better to a PEGylated recombinant human hyaluronidase (PEGPH20) based therapy 60. Further, in the adjuvant setting, the human equilibrative nucleoside transporter 1 (hENT1) has been shown to be a predictive marker in gemcitabine but not 5-fluorouracil-treated patients 106. Stromal SPARC expression had initially been suggested as a marker for nab-Paclitaxel response 35; however, no significant associations between SPARC levels and efficacy have been shown 57. Recently, large-scale genomic analyses have revealed several potential targets and subgroups. Most notably, the unstable subtype of pancreatic cancer that co-segregated with inactivation of DNA maintenance genes (see above) and a signature of DNA damage repair deficiency correlated with response to platinum-based therapies or PARP inhibitors 107-109. The very small percentage of patients with BRAF (and not KRAS) mutations might benefit from BRAF inhibitors 110. Further, transcriptome classification revealed an immunogenic subtype with upregulation of CTLA4 and PD1 that might be targeted by immune modulation therapies 111.

It remains a challenge in the future to define pancreatic cancer subpopulations, for whom specific therapies are available and deliverable and to test this approach within well designed, cost effective trials.

**Figures**

Figure 1: The pancreatic tumour microenvironment contains an abundant fibrotic stroma which includes a variety of cell types as well as extracellular matrix components, such as collagen, fibronectin, and hyaluronic acid.

Figure 2: Cancer cells express PD-L1 and cause an increase in other PD-L1 cells in the cancer microenvironment (shaded grey). PD-L1 inhibits T-Effs. CTLA4 directly inhibits T-Effs and causes APCs and MDSCs to produce kynurenine, which is also immunosuppressive. Differentiation of macrophages to the M2 form also contributes to immunosuppression. Vaccines and modified immune cells can be administered to patients to improve immune response. Indigenous T-cells, macrophages, APCs and myeloid derived suppressor cells all offer targets for immunotherapy: activation of stimulatory proteins or inhibition of suppressive proteins.

**Tables**

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| **Name** | **R0/R1** | **Treatment** | **Patients** | **Median survival** | **5 year overall survival (95% CI)** |
| **GITSG** | R0 | Observation | 22 | 11 | nd |
|  |  | 5-FU-based radiation, followed by 5-FU | 21 | 20  (p=0.035) | nd |
| **EORTC\*** | R0/R1 | Observation | 54 | 12.6 | 10 (0-20) % |
|  |  | 5-FU-based chemoradiation | 60 | 17.1 | 20 (5-35) %  p=0.099 |
| **RTOG 97-04** |  | 5-FU, 5-FU-based radiation, 5-FU | 230 | 16.9 | nd |
|  |  | Gemcitabine, 5-FU-based radiation, gemcitabine | 221 | 20.5  (p=0.9) | nd |
| **ESPAC-1**  **(all patients)** | R0/R1 | No chemoradiotherapy | 178 | 16.1 | nd |
|  |  | Chemoradiotherapy | 175 | 15.5  p=0.235 | nd |
|  |  | No chemotherapy | 235 | 14.0 | nd |
|  |  | Chemotherapy | 238 | 19.7  p=0.0005 | nd |
| **ESPAC-1**  **(2x2 only)** | R0/R1 | No chemoradiotherapy | 144 | 17.9 | 19.6% |
|  |  | Chemoradiotherapy | 145 | 15.9  p=0.05 | 10.8 (6.1 – 17.0) % |
|  |  | No chemotherapy | 142 | 15.5 | 8.4 (3.8 – 14.1) % |
|  |  | Chemotherapy | 147 | 20.1  p=0.009 | 21.1 (14.6 – 28.5) % |
| **CONKO-001** | R0/R1 | Observation | 175 | 20.2 | 10.4 (5.9 -15.0) % |
|  |  | Gemcitabine | 179 | 22.8 | 20.7 (14.7 -26.6) %  p=0.01 |
| **ESPAC-3** | R0/R1 | Gemcitabine | 539 | 23.6 | 17.5 (14.0 – 21.2) % |
|  |  | 5-FU | 551 | 23.0 | 15.9 (12.7 – 19.4) % p=0.39 |
| **CONKO-005** | R0 | Gemcitabine | 217 | 26.5 | 19% |
|  |  | Gemcitabine + erlotinib | 219 | 24.6  ns | 28% |
| **IMPRESS**  **NCT01072981** | R0/R1 | Gemcitabine +/- radiochemotherapy | 722 | 30.4 | nd |
|  |  | Gemcitabine +/- radiochemotherapy  + Algenpantucel-L |  | 27.3  ns | nd |
| **JASPAC-01** | R0 (R1)§ | Gemcitabine | 190 | 25.5 | 24.4 (18.6-30.8) % |
|  |  | S-1 | 187 | 46.5 | 44.1 (36.9-51.1) %  p<0·0001 |
| **ESPAC-4** | R0/R1 | Gemcitabine | 366 | 25.5 | 16.3 (10.2 – 23.7) % |
|  |  | Gemcitabine and Capecitabine | 365 | 28.0 | 28.8 (22.9 – 35.2) % p=0.032 |
| **RTOG 8048** |  | Gemcitabine (+/- Erlotinib #) +/- Chemoradiotherapy | 952 |  |  |
| **APACT** |  | Gemcitabine +/- Nab-Paclitaxel | 800 |  |  |
| **PRODIGE/ UNICANCER** |  | mFOLFIRINOX vs Gemcitabine | 490 |  |  |

Table 1: RCTs of adjuvant therapy for pancreatic cancer.

ns: not significant, nd: not determined/not stated

\*only pancreatic head cancers, #this arm has been closed, §R0 stated as inclusion criteria; however 13% R1 cases included

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Local ablative therapy | Method of action | Laparotomy | Percutaneous/ EUS | Invasive |
| Stereotactic body radiation | Radiation, apoptosis and non-apoptotic cell death | - | - | - |
| Radiofrequency ablation | Thermal damage: heat, coagulative necrosis | + | (+) | + |
| Irreversible electroporation | Electroporation, apoptosis | + | + | + |
| High intensity focused ultrasound | Thermal damage: heat,  coagulative necrosis | - | (+) | - |
| Photodynamic therapy | Non-thermal cytotoxic effect, necrosis | - | + | + |
| Microwave ablation | Thermal damage: heat,  coagulative necrosis | + | (+) | + |
| Cryoablation | Thermal damage: cold,  necrosis | + | (+) | + |
| Iodine-125 seed implantation | Radiation, apoptosis and non-apoptotic cell death | (+) | + | + |

Table 2: Local ablative therapies for pancreatic cancer.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Study** | **Year (published)** | **Regimes** | **Patients** | **Median survival (months)** | **p-value** |
| CONKO-003 | 2011 | Oxaliplatin, 5-FU/FA | 23 | 4.8 | 0.008 |
|  |  | BSC | 23 | 2.3 |  |
| **CONKO-003** | **2014** | Oxaliplatin, 5-FU/FA | 76 | **5.9** | 0.01 |
|  |  | 5-FU/FA | 84 | 3.3 |  |
| PANCREOX | 2014  (abstract) | FOLFOX6 | 54 | 6.1 | 0.02 |
|  |  | 5-FU/FA | 54 | 9.9 |  |
| **NAPOLI-1** | **2016** | Nanoliposomal irinotecan  plus 5-FU/FA combination | 117 | **6.1** | 0.012 |
|  |  | 5-FU/FA  combination therapy control | 119 | 4.2 |  |
|  |  | Nanoliposomal irinotecan  monotherapy | 151 | 4.9 | 0.94 |
|  |  | 5-FU/FA  monotherapy control | 149 | 4.2 |  |
| NCT01954992 |  | Glufosfamide vs. 5-FU/FA | 480 |  |  |
| NCT02506842 |  | Nab-Paclitaxel, Gemcitabine vs. Oxaliplatin, 5-FU/FA | 300 |  |  |

Table 3: Phase III RCTs of second line chemotherapy for pancreatic cancer

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Study** | **Year (published)** | **Regimes** | **Patients** | **Median survival (months)** |
| Phase II RCT  105 | 2015 | Capecitabine +/- ruxolitinib | 64/63  31/29\* | 4.5 vs. 4.3 (ns)  2.7 vs. 1.8 (p=0.011)\* |
| Phase II RCT 112 | 2015  (abstract) | mFOLFOX vs. MK-2206 and selumetinib | 60/53 | 7.5 vs. 4 (ns) |
| Phase II RCT  113 | 2014 | S1 +/- folinic acid | 45/47 | 6.3 vs. 5.5 (ns) |
| Phase II  114 | 2015 | Lapatinib and capecitabine | 17 | 5.2 |
| Phase II  115 | 2014 | Vatalanib | 67 | ~4 |
| Phase II  116 | 2014 | Capecitabine and docetaxel | 43 | 5.3 |
| Phase II  117 | 2014 | PHY906 and capecitabine | 25 | 5.0 |

Table 4: Recent phase II trials of second line (chemo)therapy for pancreatic cancer. \* in the patients with a C-reactive protein (CRP) level above the median of the study population (13 mg/L).

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