

Is metastatic pancreatic cancer an untargetable malignancy?

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Abstract

Metastatic pancreatic cancer (MPC) is one of the most aggressive malignancies, known to be chemo-resistant and have been recently considered resistant to some targeted therapies (TT). Erlotinib combined to gemcitabine is the

only targeted therapy that showed an overall survival benefit in MPC. New targets and therapeutic approaches, based on new-TT, are actually being evaluated in MPC going from immunotherapy, epigenetics, tumor suppressor gene and oncogenes to stromal matrix regulators. We aim in this paper to present the major causes rendering MPC an untargetable malignancy and to focus on the new therapeutic modalities based on TT in MPC.

Key words: Pancreatic cancer; Tumor suppressor genes; Targeted therapies; Immunotherapy; Epigenetics

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Core tip: This paper will report on the most recent updates in the treatment of metastatic pancreatic cancer (MPC). We present the major causes rendering MPC an untargetable malignancy and we focus on the new therapeutic modalities based on targeted therapies in MPC.

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INTRODUCTION

Pancreatic cancer (PC) is one of the most aggressive and devastating solid tumors, with less than 5% of patients still alive at 5 years^[1]. Its poor prognosis is also due to the late diagnosis of PC and the absence of early detection tools or markers.

The incidence of this cancer is increasing worldwide; it represents actually the seventh most diagnosed cancer in Europe and the fifth leading cause of cancer mortality^[2]. More than 80% of these cancers are locally advanced

or metastatic at diagnosis. The only curative treatment remains surgery, possible in less than 20% of the patients with PC, diagnosed at an early stage^[1].

Adenocarcinomas represent the majority of PC, less than 5% are neuroendocrine tumors. Pancreatic neuroendocrine tumors have specific features and different treatment modalities^[3]. In this paper, we will focus only on metastatic pancreatic adenocarcinomas.

Many risk factors and inherited syndromes are incriminated in the development of this cancer. PC represents a genetically complex and heterogeneous tumor; it results from successive accumulation of gene mutations. PC tumor cells harbor more than 60 genetic alterations that affect more than twelve signaling pathways^[4].

According to the national cancer institute dictionary, targeted therapies (TT) are defined as a type of treatment that uses drugs or other substances to identify and attack specific types of cancer cells with less harm to normal cells^[5]. Till the end of the last century, hormonal therapies were considered one of the rare routinely used drugs respecting the definition of TT in oncology. After hormonal therapies, monoclonal antibodies and tyrosine kinase inhibitors (TKIs) were widely being tested and approved in the management of different cancers during the last 15 years.

TT can be divided into two major groups: Therapies targeting the cancer cell itself and therapies targeting the tumor microenvironment. The first group includes hormonal therapies, small molecules (TKIs) and monoclonal antibodies; antibody drug conjugates and oncolytic viruses were recently added to this category. The second group includes mainly angiogenesis inhibitors and immunotherapy.

In the emerging era of TT, this new concept was widely introduced in the management of different tumors; however, PC remains resistant to available TT. Is metastatic pancreatic cancer (MPC) an untreatable malignancy? This review focuses on the major clinical trials evaluating TT in MPC and the potential new targets and new approaches in MPC.

APPROVED TREATMENTS IN MPC

Before Gemcitabine era in PC, 5-FU was the most frequently used chemotherapy in the treatment of MPC. In 1997, gemcitabine, as monotherapy, was approved by the Food and Drug Administration (FDA) as the first line treatment in MPC after a trial comparing gemcitabine to 5-FU in untreated MPC; less than two months of difference in overall survival were good enough for the approval of gemcitabine as a first line therapy in MPC^[6]. During 14 years, two new combinations associating gemcitabine with either erlotinib or cisplatin were considered potential regimens in the treatment of MPC. The first was approved based on a minimal overall survival benefit when added to gemcitabine compared to gemcitabine alone, knowing that this regimen is only beneficial to the subgroup of patients that develop rash^[7], while the approval of the second regimen was based on

two meta-analyses^[8,9].

The turning point in the natural history of MPC was in 2011 with the approval of the FOLFIRINOX regimen. FOLFIRINOX was the first chemotherapeutic regimen that surpasses the barrier of one year of survival in MPC. It became the standard of care in patients having MPC with good performance status. The major side effects of this regimen are neutropenia, fatigue diarrhea and sensory neuropathy^[10]. Another regimen, associating gemcitabine with nab-paclitaxel, showed a better overall survival when compared to gemcitabine alone, and was therefore approved in the treatment of MPC. Its toxicity profile was different compared to FOLFIRINOX: Less febrile neutropenia and fatigue and more peripheral neuropathy^[11].

TT IN MPC

After the approval of gemcitabine as standard of care in MPC in 1997 and the emergence of TT during the same period, many trials tried to associate gemcitabine to one of the TT. All these trials had negative results; only Gemcitabine-erlotinib combination demonstrates statistically significantly improved survival in advanced PC^[7]. Although the practical implication and benefit of the use of this drug remains debatable, erlotinib is currently the only FDA approved targeted therapy drug for pancreatic adenocarcinoma.

The results phase III trials associating TT to gemcitabine were disappointing. Thus, cetuximab^[12], bevacizumab^[13], aflibercept^[14], axitinib^[15], sorafenib^[16] and ganitumab^[17] failed to show any benefit when added to gemcitabine and compared to gemcitabine alone in the treatment of MPC. The results of these studies (PFS-OS) and mechanisms of action of TT are summarized in the Table 1.

Associating two TT with gemcitabine also failed to show any positive results, the association of erlotinib and bevacizumab to gemcitabine was not superior to erlotinib-gemcitabine^[18]. Another phase I trial evaluated the combination of gemcitabine-erlotinib-cixitumumab, but without any promising results^[19]. Associating two chemotherapies (gemcitabine and capecitabine) and two TT (bevacizumab and erlotinib) showed promising results with an overall survival exceeding one year in a phase I / II trial^[20]. Clinical trials in MPC associating two TT to chemotherapeutic agents are summarized in the Table 2. Many TT are tested as single agent in second line treatment of MPC; selumetinib (anti-MEK) and everolimus (mTOR inhibitor) are two examples^[21,22]. Combining two TT without chemotherapy is also being tested actually in new ongoing trial in a second line treatment of MPC with afatinib and selumetinib compared to capecitabine (NCT02450656) and sorafenib plus everolimus (NCT 00981162).

MECHANISMS OF RESISTANCE OF PC TO CHEMOTHERAPY AND TT

PC was known to be resistant to chemotherapy before

Table 1 Phase III trials evaluating gemcitabine with a targeted therapy in advanced or metastatic pancreatic cancer

Ref.	Regimen	Mechanism of action	PFS	OS
Moore <i>et al</i> ^[7]	Gemcitabine/erlotinib	Anti-EGFR	3.7	6.2
	Gemcitabine		3.5	5.9
Philip <i>et al</i> ^[12]	Gemcitabine/cetuximab	Anti-EGFR	3.4	6.3
	Gemcitabine		3.0	5.9
Fuchs <i>et al</i> ^[17]	Gemcitabine/ganitumab	Anti-IGF1R	3.7	7.2
	Gemcitabine		3.6	7.0
IokaT <i>et al</i> ^[15]	Gemcitabine/axitinib	TKI	NA	5.1
	Gemcitabine		NA	5.4
Gonçalves <i>et al</i> ^[16]	Gemcitabine/sorafenib	TKI	5.7	9.2
	Gemcitabine		3.8	8.0
Kindler <i>et al</i> ^[13]	Gemcitabine/bevacizumab	Anti-VEGF	3.8	5.8
	Gemcitabine		2.9	5.9
Rougier <i>et al</i> ^[14]	Gemcitabine/aflibercept	Anti-VEGF	3.7	6.5
	Gemcitabine		3.7	7.8

PFS: Progression free survival; OS: Overall survival; EGFR: Epidermal growth factor receptor; IGF1R: Insulin-like growth factor receptor 1; TKI: Tyrosine kinase inhibitor; VEGF: Vascular endothelial growth factor; NA: Not available.

the era of FOLFIRINOX; many hypotheses tried to elucidate this chemo-resistance. Thus, alterations in key pathways involved in cell cycle control, namely apoptosis, were largely incriminated. NF κ B, pro-inflammatory and anti-apoptotic factor, seemed to be a key link between inflammation and cancer chemo-resistance in PC. The genetic complexity and heterogeneity of PC is also a challenge in the treatment of this cancer, since more than 60 genetic alterations affecting more than twelve signaling pathways are involved in its pathogenesis. The most commonly affected signaling pathways in PC are apoptosis, DNA damage repair, G1/S transition (CDKN2A/p16, CyclinD), cell-cell adhesion, regulation of invasion, embryonic signaling (Notch pathway, Hedgehog pathway and Wnt pathway) and MAPK signaling [c-Jun N-terminal kinase, ERK and transforming growth factor beta (TGF- β) signaling]^[23].

Actually, recent data revealed that tumor stroma is a major extrinsic mechanism of resistance to chemotherapy and targeted therapy of PC. The tumor stroma of PC is limiting the drug delivery to pancreatic tumor cells at therapeutically relevant concentrations^[24].

Next to intrinsic resistance to TT due to the multitude of pathways incriminated in the pathogenesis of PC, extrinsic resistance seems to be as important in the mechanism of non-response to TT. This extrinsic resistance seems to be particular to PC, rendering it a hardly targetable malignancy.

NEW TREATMENT APPROACHES AND TARGETS IN MPC

PC represents one of the least targetable malignancies with the present available drugs. Despite the huge efforts in research and promising results in some phase I and II trials, TT have not been approved yet in this

Table 2 Clinical trials in metastatic pancreatic cancer associating two targeted therapies to chemotherapeutical agents

Ref.	Regimen	Phase	PFS	OS
Van Cutsem <i>et al</i> ^[18]	Gemcitabine/erlotinib/bevacizumab	III	4.6	7.1
	Gemcitabine/erlotinib		3.6	6.0
Philip <i>et al</i> ^[19]	Gemcitabine/erlotinib/cixutumumab	I	3.6	7.0
	Gemcitabine/erlotinib		3.6	6.7
Watkins <i>et al</i> ^[20]	Gemcitabine/capecitabine/erlotinib/bevacizumab	I / II	8.4	12.6
	Afatinib/selumetinib		II	NA
NCT02450656 (ongoing trial)		Capecitabine		

PFS: Progression free survival; OS: Overall survival; NA: Not available.

indication, neither influenced the natural history and the evolution of PC. New approaches and modalities are necessary to counteract the resistance to treatment of this malignancy, from immunotherapy and epigenetics to oncogenes and tumor suppressor genes regulation (Table 3).

Immunotherapy

Several immunotherapy approaches for PC have shown promising results in early clinical trials. Checkpoint inhibitors represent actually the most expanding and booming novel therapeutic approach in oncology; many new agents were recently approved in melanomas^[25-27] and lung cancer^[28]. Adoptive T cell transfers are also being largely evaluated and studied with encouraging results in some cancers including PC.

Many trials are testing different checkpoint inhibitors in advanced solid tumors including MPC. Check point inhibitors are new immunologic agents, that block inhibitory receptors of immune system elements, such as cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), programmed cell death protein -1 (PD-1) and its ligand PD-L1, leading to the activation of tumor-specific T cells with effector function against tumor cells^[29]. An ongoing phase I trial is evaluating a checkpoint inhibitor exclusively in advanced PC by combining gemcitabine and ipilimumab (anti-CTLA4).

Adoptive cell therapy is a new therapeutic approach based on the modification and selection of autologous T cells *in vitro* from the patient's tumor in order to have more potent and efficient T-cells, either by collecting tumor infiltrating lymphocytes (TILs) or by developing genetically engineered cells. These modified and selected T cells are re-infused to the patient. Two different mechanisms are implicated in the concretization of the concept of genetically engineered cells, the first by modifying genetically a T-cell receptor for cancer antigen (transgenic TCR) and the second by adding a chimeric antigen receptor (CAR), which recognizes a specific cancer antigen^[30]. Two specific phase I / II trials for PC are adopting this new approach. The first one is a phase I clinical trial exploring the potential of CAR T cells modified to recognize mesothelin, which is expressed in all PCs but not in healthy pancreatic cells (NCT01897415,

Table 3 New treatment modalities based on targeted therapies in metastatic pancreatic cancer

Treatment modality	Mechanism of action	Target
Immunotherapy	Check point inhibitors	CTLA4
	Adoptive cell therapy	T-cells
Epigenetics	Histone acetylation	Histones
Stromal	Hyaluronidase	Hyaluronan
extracellular matrix		
Tumor suppressor genes regulation	miRNA inhibitors	TP53-SMAD4- CDKN2A
	Anti-PARP	BRCA1-BRCA2

CTLA4: Cytotoxic T-lymphocyte-associated antigen 4; TP53-SMAD4-CDKN2A: Tumor protein p53 - cyclin-dependent kinase inhibitor 2A; BRCA: Breast cancer; PARP: PolyADP ribose polymerase.

NCT02159716). The second one is a phase I /II trial also testing CAR T cells modified to recognize mesothelin in patients with PC at the National Cancer Institute (NCT01583686). Many other trials including patients with solid tumors (including PC) are evaluating the effect of reinfusing TILs (NCT01174121) and genetically reengineered T cells to target the NY-ESO-1 antigen in patients with NY-ESO-1-positive cancers (NCT01967823).

Toll-like receptors (TLRs) recognize distinct pathogen-associated molecular patterns and play a critical role in innate immune responses. They participate in the first line of defense against invading pathogens and play a significant role in inflammation, immune cell regulation, survival, and proliferation. Likewise, TLRs can start immunological reactions against endogenous molecules released into the extracellular compartment under due to stress or tissue damage^[31]. TLRs are expressed in the PC tissue, whereas they are not expressed in the normal pancreas^[32,33]. Thus, they appear to play a role in the pathophysiology of PC and may thereby also represent targets for intervention. Many ongoing phase I /II trials combining TLRs with chemotherapy or radiation therapy are being evaluated in breast cancer, sarcomas and melanomas are being evaluated and some preclinical trials in PC showed promising results^[34].

Epigenetics

Epigenetic modifications are independent from the changes in the DNA sequence. They are due to variations in DNA methylation and histone acetylation. Many new drugs, targeting methylation of DNA and acetylation of histones have been mainly approved in hematology. This new approach is also being evaluated in PC. Ongoing phase I and II trials in PC are associating vorinostat, gemcitabine, bortezomib (anti-proteasome) and radiation therapy (Clinical Trial.gov. NCT00983268, NCT00243100).

Targeting tumor suppressor genes and oncogenes

As mentioned before, PC is caused by multiple genetic sequential changes. This subtype of cancer is associated also with chromosomal instability, since more than 90% of PC is aneuploidy: Presence of losses and gains of large portions of chromosomes or whole chromosomes

leading to abnormal karyotype. The accumulation of genetic instability mainly higher number of mutations of oncogenes and tumor suppressor genes is a part of the early event of the development of PC. More than 80% of ductal PCs exhibit KRAS mutations. Furthermore, 90% of the tumors exhibit deletions, mutations or epigenetic alterations in the *CDKN2A* gene. Nearly 50% have mutations in the tumor suppressor p53 and also approximately 50% exhibit mutations or homozygous deletions in the *SMAD4* gene^[2]. Ten percent of sporadic PCs present one of these mutations present BRCA1 or BRCA2 mutations^[35].

MicroRNAs, single-stranded chains of non-coding RNA of small number of nucleotides, that inhibit gene expression at the post-transcriptional level, regulate CDK2NA, TP53 and SMAD4 tumor suppressor genes^[36]. For example, CDK2NA or p16 inhibits cycline -dependant kinase 1, 4 and 6 and also help to stabilize p53; CDKN2A itself is regulated by a microRNA, miR-10b^[37]. TGFβ is a potent tumor suppressor that signals *via* the SMAD pathway and intersects with the WNT beta-catenine signaling pathway; it regulates the cell cycle by inhibiting cyclin-dependant kinases, E2F and histone deacetylase during the G1 phase of the cell cycle. TGFβ itself is regulated by different miRNA including mi-RNA 15/16 and mi-RNA 224^[38,39].

Some of these microRNAs inhibiting these tumor suppressor genes are in relation with the resistance to chemotherapy (gemcitabine), the poor prognosis of PC and the higher potential to rapid progression and metastasis development; inhibiting these microRNAs can be a promising strategy in TT. Other microRNAs are in relation with radio-resistance of PC; in a recent study evaluating a radio-resistant pancreatic cell line, miRNA-216a was significantly down regulated, whereas the autophagy activity was controlled. Using bioinformatics analysis, it was concluded that forced expression of microRNA-216a enhances the radio-sensitivity of pancreatic cells by inhibiting beclin-1 mediated autophagy^[40].

BRCA1 and *BRCA2* are two genes implicated in the DNA repair process. A mutation in one of these genes can cause breast, ovarian, and PCs. Recently another gene of the same family, the *PALB2*, was incriminated in the development of PC^[41]. Many studies are evaluating anti-PARP drugs in patients carrying BRCA1 and BRCA2 diagnosed with breast and ovarian cancers with promising results. Anti-PARP drugs cause multiple double strand breaks in the DNA and in tumors carrying one of these three mutations, these DNA breaks cannot be efficiently repaired, leading to the death of the cells. The FDA has recently approved Olaparib (anti-PARP) in BRCA-mutated ovarian cancers^[42]. Thus, the presence of one of these mutations can be a predictive biomarker of the use of these new drugs alone or in combination with gemcitabine in patients with PC. Many ongoing trials are studying this treatment options. NCT00515866 trial is evaluating the safety and tolerability of a PARP inhibitor in combination with gemcitabine in PC. Another randomized, phase II trial (NCT01585805) is testing the veliparib (anti-PARP) in

combination with gemcitabine hydrochloride and cisplatin compared to gemcitabine hydrochloride and cisplatin alone in patients with pancreatic adenocarcinoma having a known BRCA/PALB2 mutation.

Targeting stromal extracellular matrix

One of the important reasons of resistance to treatment in PC is the difficulty to deliver drug to tumor cells, because of the extensive deposition of extracellular matrix components and low vascularization of tumor environment. Targeting stromal extracellular matrix components seems to be an interesting approach to counteract this mechanism of resistance. Many trials targeting matrix metalloproteinases failed to show any benefit^[43,44], actually new targets are being tested using Hyaluronidase next to gemcitabine with promising results^[45]. Hyaluronidase acts by depleting pancreatic tumors of their high hyaluronan content in preclinical trials; hyaluronan being a glycosaminoglycan, one of the major components of extracellular matrix throughout the pancreatic tumor.

Targeting oxidative stress, chronic inflammation and targeting programmed cell death pathway

Oxidative stress has been shown to participate in the process of PC. Evidences supporting the role of reactive oxygen species and cytokines, as factors in the development of PC have been proposed. The concept of antioxidant supplementation as a preventive approach for PC has been evaluated^[46]. Curcumin, resveratrol, and genistein have antioxidant activities and demonstrated anti-cancer effects against PC *in vitro* and *in vivo* experiments^[47-49].

Chronic inflammation seems also to be incriminated in the development of PC. Many studies showed an increased incidence of pancreatic in patients with chronic pancreatitis. Prolonged inflammation may precede the onset of frank malignancy by a significant interval, and that once malignancy is established, the resulting inflammation occurred may act as a continued driving force in accelerating further malignant change. Targeting this chronic inflammation may prevent or postpone the process of PC. NFκB, cyclooxygenase 2 (COX2), lipoxygenase and inducible nitric oxid (NO) are the main targetable components of the chronic inflammation in PC^[50]. A study of 28283 participants, over a 7-year period, found that women who took regular aspirin had a 43% lower risk of PC than women who did not use aspirin^[51].

PC is characterized by an important resistance to apoptosis, which is associated by high expression levels of multiple prosurvival proteins of the extrinsic and intrinsic apoptosis signaling cascades and/or reduced expression or function of pro-apoptotic proteins. Many components of programmed cell death signaling pathways are being studied as targets for cancer therapies, for example the TRAIL system, IAP proteins or anti-apoptotic Bcl-2 proteins^[52].

DISCUSSION

The molecular and genetic complexity of MPC is one

of the major barriers causing the failure of TT in this indication; more than 60 genetic alterations that affect more than twelve signaling pathways are involved in the development of PC rendering this tumor resistance to TT. Inhibiting one pathway by a specific targeted therapy is not sufficient to block to the cellular proliferation and will probably induce the activation of another pathway. Many other factors make MPC difficult to treat: The aggressive molecular and cellular features, the late diagnosis and absence of tools of early detection the stromal proliferation forming a drug's barrier, the reduced vascular density and the immune suppression.

Detecting PC at an early stage remains the most rationale and solid perspective in the future management of this disease. At present, serum CA-19-9 (carbohydrate antigen 19-9) is the only FDA-approved biomarker for PDA, and it has utility marker of disease recurrence and surveillance. There has been a recent explosion in the PC biomarker field with more than 2000 biomarker studies implicating thousands of informative genes as candidate biomarkers^[53].

Many markers of early detection of PC are being evaluated in blood, pancreatic cyst fluid, pancreatic juice and stool based on the new advances in technology for whole genome, methylome, ribonucleome and proteasome interrogation. Many promising results are being reported for different markers of early detection of PC^[54]. Circulating tumor cells are one of the promising markers in blood used to the early detection of PC; the detection of a mutation, as KRAS for example, in the cells of pancreatic juice can help the early diagnosis of the PC^[55,56]. Glycpan-1 circulating exosomes were detected in the serum of patients with PC with absolute specificity and sensitivity; this new diagnostic and screening marker may serve as a potential non-invasive tool to detect early stages of PC and consequently, to facilitate possible curative surgical therapy^[57]. Recently, a new non-invasive urinary biomarker, based on a set of three urinary proteins (LYVE-1, REG1A, and TFF1) was identified, able to distinguish patients with early-stage PDAC from healthy individuals^[58].

To conclude, associating many TT, based on well-defined molecular biomarkers leading to a specific profile, can surpass some of those obstacles aiming to offer the best treatment to the appropriate patient. Combining different approaches in the same patient according to his molecular profile can be the best treatment option.

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