Translational Research in Radiotherapy of Pancreatic Adenocarcinoma: A Review

Abstract
Cancer of the pancreas is one of the tumors with the worst prognosis. Radiation therapy has been proposed as an exclusive or adjuvant/neoadjuvant treatment. However, its effectiveness is limited by the low tumor radiosensitivity and by the frequent and early hematogenous metastasis. In order to improve the results of radiotherapy, various protocols of concurrent chemoradiation have been extensively tested. The clinical results have been disappointing and the role of concurrent radio-chemotherapy combination is widely debated.

More recently, a series of preclinical studies attempted to identify potential targets in pancreatic cancer to allow individualized treatments, to increase radiosensitivity and, more generally, to improve effectiveness of radiation therapy. In this context, EGFR, KRAS, p53, and ATDC have been studied as potential targets. While cetuximab, nelfinavir, recombinant adenovirus-p53 and cyclopamine were tested as potential targeted therapies.

Although most of published studies are preliminary (phase I or phase II), they produced some promising results and therefore larger phase III trial can be justified. It is possible, in the future, that the standard management of pancreatic carcinoma will be represented by multi-modality treatments combining conventional therapeutic strategies (chemotherapy, chemo-radiation) with novel targeted drugs.

Key words: Radiotherapy; Translational research; Pancreatic neoplasms; Review

Introduction
The prognosis of pancreatic adenocarcinoma (PAC) is so unfavorable that the annual incidence and mortality rates of this disease are equivalent [1]. PAC is currently the fourth leading cause of cancer mortality in the United States but in 2030 forecasts it represents the second leading cause of cancer mortality with an extremely low 5-year overall survival [2,3]. Particularly, in 2013 over 45,000 new cases of PAC were diagnosed in the United States [2] and of these only 20% presented with resectable disease [4-7].

Actually the only chance of cure in PAC is radical surgery with tumor free margins [5,8,9]. However, only in a small percentage (20%) of highly selected patients is a complete surgical resection feasible [10]. In fact, about 40% of patients with PAC present at diagnosis with inoperable locally advanced disease [11]. Furthermore, even in patients undergoing radical surgery, 5-year survival remains about 15-20% with 30% mortality rate due to local recurrence [11].

Although the cure rates of PAC are marginally improved by the use of adjuvant therapies, definition of a standard approach for the treatment of locally advanced disease is currently not available [12]. The international guidelines suggest a multidisciplinary approach based on different multimodal strategies (chemotherapy alone or chemoradiation or both) [13,14]. An emerging trend is represented by the possibility to adapt the treatment based on patients characteristics (performance status, age, comorbidities) [14] and tumor characteristics (stage, grading and genetic profile of their disease) [15]. Anyway, despite recent advances, the prognosis of patients affected by PAC is dismal, highlighting the need for new therapeutic approach based on preclinical and translational studies.

In the last decades progress in the research on PAC biology have
led to cancer microenvironment becoming a target for therapies [12,16]. In fact, tumor proliferation can be modulated by the stromal compartment regulating blood supply and hypoxia with the potential of enhancing the chemotherapy delivery to the tumor.

The principal cause of treatment failure in PAC is the intrinsic chemoresistance and radioresistance [17]. This feature is mostly due to the presence of tumor hypoxic regions significantly correlated with worst prognosis. Several mechanism (apoptosis inhibition, angiogenesis stimulation, up-regulation of genetic and epigenetic changes) can increase hypoxia hence increasing even tumor invasion and resistance to treatments [18]. With the aim to overcome tumor resistance to standard treatments, some new biological therapies are targeting the stroma [19-24], the Epidermal Growth Factor Receptor (EGFR) [25] or the Vascular Endothelial Growth Factor Receptor (VEGFR).

The biology of PAC having gained a better understanding, translational research is now directed towards identification of novel and effective agents in curative and adjuvant therapies [12,26]. On the basis of these premises, the aim of this review is to briefly summarize the main evidences in the field of translational research in radiotherapy treatments of PAC.

Preclinical and Clinical Studies

The EGFR is overexpressed in over 90% of pancreatic cancer samples [27,28]. Based on this high rate, several studies were carried out to evaluate the possibility of selectively targeting this receptor. A phase II trial enrolled 21 patients with advanced PAC receiving a cetuximab (a chimeric monoclonal antibody inhibiting the EGFR) loading dose (400 mg/m2) and a weekly cetuximab dose (250 mg/m2) during RT (50.4 Gy in 28 fractions) was published [25]. An ancillary feasibility study of on-trial patient blood and skin sampling was also performed. The results reported were grade ≤ 2: acute toxicity: 70%, median overall survival: 7.5 months, 1-year actuarial survival: 33% and 3-year overall survival: 11%. The authors concluded that cetuximab favors PAC radiosensitization by achieving better tumor control with marginal toxicity. Before this study, in 2005 [29] and 2011 [30], other authors demonstrated the stimulating effect of EGFR in repairing radiation-induced DNA damage. Patient skin and blood samples yielded sufficient RNA and proteins, which encouraged new studies on future biomarker analysis with the aim to predict individual tumor response [25].

Some studies in PAC demonstrated that EGFR signaling is required for KRAS driven pancreatic tumorigenesis [31,32] and that KRAS is widely mutated in PAC (80-100% of cases) [33,34]. In 2009 an American group [35] prospectively evaluated plasma KRAS as a potential marker of response in 12 patients with locally advanced pancreatic cancer receiving induction gefitinib (an EGFR tyrosine kinase inhibitor) and chemoradiotherapy (paclitaxel concurrent to radiotherapy). KRAS codon 12 mutations confirmed by direct sequencing were detected using a two-stage restriction fragment length polymorphism-polymerase chain reaction assay on patients’ plasma both before and after therapy. Five patients presented KRAS mutations pretreatment. In 3 of those KRAS was undetectable post-treatment and these patients showed prolonged overall survival (8, 11 and 21 months) compared to patients who retained KRAS after treatment (2 and 4 months). Therefore, changes in KRAS plasma level may predict response to treatment. A trial based on KRAS targeting with an inhibitor failed to show clinical results [36], probably because no available effective targeting of KRAS is possible due to its intrinsic mode of action based on current knowledge.

The efficacy of radiotherapy in PAC is limited by the low radiation doses generally used, due to the risk of toxicity particularly in patients treated with concurrent chemoradiation [25]. Therefore, some studies were directed towards the association of molecular agents with radiotherapy to intensify response and minimize toxicity. In 2008 [37] a group from Oxford conducted a phase I trial on the association of nelfinavir to chemoradiotherapy in 12 patients with borderline resectable or unresectable PAC. The rational of this study was the mediation role of PI3 kinase for EGFR and KRAS from preclinical studies. Nelfinavir is a protease inhibitor for PI3 kinase that potentially may have radiosensitizing effects [38-40]. In 5 of 10 patients who completed chemoradiotherapy the authors observed partial CT responses, with 5 complete PET responses and 6 R0 resection. The median survival was 18 months with an acceptable non haematological toxicity. Now a Cancer Research UK group (Clinicaltrials.gov NCT02024009) is conducting a phase II clinical randomized trial (SCALOP 2) on nelfinavir which started in April 2015. Patients are treated with induction chemotherapy (gemcitabine and nab-paclitaxel) and those obtaining a stable disease will be randomized to receive the same regimen of induction chemotherapy, conventional chemoradiotherapy (capecitabine with radiotherapy at low doses), high doses chemoradiotherapy (capecitabine with radiotherapy at high doses), low doses chemoradiotherapy with nelfinavir or high doses chemoradiotherapy with nelfinavir.

In over 75% of the pancreatic carcinoma p53 oncosuppressor, promoting DNA repairing from radiation damage, is mutated thus increasing tumor radioresistence [41,42]. In 2011 a Chinese group assessed the efficacy of the combination of chemoradiotherapy (Intensity Modulated Radiotherapy plus gemcitabine) and recombinant adenovirus-p53 (rAd-p53) in 15 patients with unresectable pancreatic carcinoma. In this pilot study, target therapy with rAd-p53 showed encouraging preliminary results in terms of survival and toxicity [43] (Table 1).

Furthermore, other strategies has been used to improve tumor radio-sensitivity by targeting different molecules. In 2013 a Chinese preclinical study analyzed cyclopamine, an hedgehog-inhibitor, as radiosensitizing agent on tumor pancreatic cells through a KRAS-independent pathway [44]. In 2014 an American group published the results of an in vitro and in vivo research on ATDC/TRIM29, a binding protein of DNA enhancing tumor growth [45]. It was found that ATDC is overexpressed in PAC, playing a role on DNA damage resulting in increased tumor radioresistence. More interestingly, both in vitro and in vivo assays, the knockdown of this protein sensitizes PAC to ionizing radiation.

Finally, another emerging and potentially useful strategy in combined modality treatment of PAC is represented by the use of nanomedicine techniques to optimize concurrent...
chemoradiotherapy. In fact, nanoparticles may deliver chemotherapy with a preferential accumulation into the tumor and with a minimale uptake in normal tissue. This makes nanoparticles an ideal way to deliver chemotherapy in radiochemotherapy regimens [46].

**Conclusions**

Nowadays, PAC remains one of the hardest challenges for clinical and translational researches. Despite some improvement in prognosis, the cost in terms of life and medical expenditures are very high. Innovative treatments facing PAC using different treatment strategies could be the best chance to make PAC a largely curable disease [47]. Progresses in translational research have surely produced some promising results so that probably, in the future, the gold standard treatment of PAC will be represented by multi-modality treatments combining conventional therapeutic strategies (chemotherapy, radiochemotherapy) with novel targeted drugs.

**Conflicts of interest notification**

No actual or potential conflicts of interest do exist regarding this paper.

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**Table 1** target therapy with rAd-p53 showed encouraging preliminary results in terms of survival and toxicity.

<table>
<thead>
<tr>
<th>Author/Year</th>
<th>Patients</th>
<th>Study design</th>
<th>Treatment</th>
<th>Main findings</th>
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<tbody>
<tr>
<td>Brunner TB et al., 2008 [37]</td>
<td>18 unresectable/ borderline resectable CAP</td>
<td>Phase I</td>
<td>RT and concurrent cisplatin + gemcitabine + nelfinavir</td>
<td>acceptable toxicity and promising activity: 5 PR at CT scan; 5 CR at PET; 2 SD; 6 RO resections with 1 complete pathological response.</td>
</tr>
<tr>
<td>Olsen CC et al., 2009 [35]</td>
<td>11 locally advanced CAP</td>
<td>Phase I</td>
<td>7-day induction gefitinib (250 mg) followed by daily gefitinib + concurrent CRT (50.4 Gy/28 fractions of RT + weekly paclitaxel, 40 mg/m2 IV) followed by gefitinib maintenance</td>
<td>daily gefitinib + concurrent CRT was reasonably tolerated. Survival times were favourable in patients with undetectable post-treatment k-ras mutations post-treatment.</td>
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<tr>
<td>Li JL et al., 2011 [43]</td>
<td>15 unresectable CAP</td>
<td>Phase I-II</td>
<td>rAd-p53 + CRT (IMRT 55-60 Gy/25-30 fx + gemcitabine 350 mg/mq weekly for 6 weeks)</td>
<td>rAd-p53 + CRT in unresectable CAP was well tolerated and showed encouraging clinical benefit response (53.3%) and disease control rate (80.0%). Median PFS and OS were 6.7 and 13.8 months, respectively.</td>
</tr>
<tr>
<td>Rembielak AL et al., 2014 [25]</td>
<td>21 locally advanced CAP</td>
<td>Phase II</td>
<td>cetuximab (loading dose: 400 mg/m2 + weekly dose: 250 mg/m2) + concurrent RT (50.4 Gy/28 fx)</td>
<td>cetuximab inhibits EGFR-mediated radioresistance but does not control metastatic progression (6 months post-treatment: 90% SD; only 33% free from metastatic progression). Median OS: 7.5 months. Extended survival was associated with high-grade acneiform rash (p=.003), post-treatment stable disease (p=.006) and pretreatment CA19.9 level (p=.004).</td>
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</table>

**Abbreviations:** CAP: Carcinoma of the Pancreas; CR: Complete Response; CRT: Chemo-Radiation; Fx: Fractions; OS: Overall Survival; PFS: Progression-free Survival; PR: Partial Response; rAd-p53: Recombinant Adenovirus-p53; RT: Radiotherapy; SD: Stable Disease.
References


Southwest Oncology Group (SWOG 9924) study. Invest New Drugs 23: 485-487.


