Immunotherapy of Pancreatic Cancer

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Ryan Stevenson, Martin Goodman, and Muhammad Wasif Saif

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R. Stevenson, MD (✉)
Department of Hematology/Oncology, Tufts University School of Medicine, Boston, MA 02111, USA
e-mail: rstevenson@tuftsmedicalcenter.org

M. Goodman, MD
Department of Hematology/Oncology, Tufts University School of Medicine, Boston, MA 02111, USA

M.W. Saif, MD, MBBS
Department of GI Oncology, Tufts University School of Medicine, Boston, MA 02111, USA
e-mail: wsaif@tuftsmedicalcenter.org

12.1 Introduction

Patients with pancreatic cancer have a very poor prognosis with it being the fourth leading cause of cancer-related death in men and women in the USA [1]. In developing countries, there were an estimated 165,100 new cases and 161,800 estimated deaths in 2011 [2]. In 2010, in the USA, 36,800 deaths were attributed to pancreatic cancer and a 5-year survival <5 % [1].

The management of patients with pancreatic cancer depends on the extent of disease at diagnosis. Surgical resection with negative margins with no lymph node involvement is the only chance for cure. The use of adjuvant chemotherapy improved survival in early-stage pancreatic cancer. The majority of patients present with locally advanced unresectable disease or distant disease, most commonly to the liver or peritoneal surface. Survival for metastatic pancreatic cancer remains poor and less than 20 % survive at the end of 1 year. There are only few chemotherapy agents that have shown an effect in pancreatic cancer including single agent gemcitabine, nab-paclitaxel with gemcitabine, and a new combination of 5-fluorouracil, leucovorin, oxaliplatin, and irinotecan (FOLFIRINOX) [3, 4, 113]. Chemoradiation has shown some benefit in locally advanced unresectable pancreatic cancers; however, it is minimal. Survival of patients with unresectable disease with these modalities is marginal, which warrants further investigation of other therapies. Immunotherapy might be an alternative treatment modality to this deadly disease.

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12.2 Evidence that Pancreatic Adenocarcinoma Elicits Immune Response

Immune-based therapy for pancreatic cancer has gained attention in every decade and, as such, generates short-lived enthusiasm. Pancreatic cancer is characterized by a highly immunosuppressive environment, with multiple components and pathways that inhibit effective pancreatic cancer-targeted immune responses. Therefore, there is great potential to target these mechanisms of immunosuppression and reverse them to create an environment that supports the infiltration of antitumor immune responses and enables the generation of T cells capable of killing pancreatic tumor cells. Each of these components and pathways represents a potential target for pancreatic cancer immunotherapy based on the supposition that the immune system can discriminate tumor cells from normal cells [5]. The data suggest that cancer patients generate B and T cells that recognize antigens expressed on autologous pancreatic tumor cells [6–12]. In addition, the animal models showed that mice deficient in genes associated with immunity (e.g., *IFN* [13] and *perforin* [14]) are susceptible to cancer development. Moreover, the analysis of immune infiltrates in human tumors has revealed a strong association between prognosis and the presence of a humoral response to pancreatic tumor antigens, such as MUC-1 and mesothelin, and of tumor-infiltrating cytotoxic T lymphocyte and Th1 cells [11, 12, 15, 16]. On the other hand, in a mouse model in which an activating K-Ras mutation is expressed in the pancreas, preinvasive pancreatic lesions are characterized by the infiltration of immune suppressor cells rather than immune effector cells, suggesting that tumor immunity may be blocked from the inception of pancreatic cancer development [17]. All mice with the K-Ras mutation develop pancreatic adenocarcinoma and eventually die of cancer. Another finding that antagonism of negative T-cell regulators, such as cytotoxic T-lymphocyte-associated (CTLA) protein-4 and B- and T-lymphocyte attenuator (BTLA) can augment the antitumor immune response further confirms that patients produce an immune response to the tumor [18, 19].

Despite the presence of the above data that underlines the fact that an antitumor immune response is elicited in cancer patients, unfortunately this response is ineffective and does not result in the killing of the tumor. Given that most tumor antigens are self- or mutated self-antigens and that the pancreatic tumor microenvironment is immunosuppressive, this is not surprising [20]. Interestingly, both the prevalence of Treg in peripheral blood and tumor, and the expression level of programmed cell death ligand 1 (PD-L1) in tumor independently predict a poor survival in pancreatic cancer [21, 22]. Tregs that constitute 5–10 % of $CD4^+$ T cells induce immune tolerance by suppressing host immune responses against self- and nonself-antigens [23–28], hence playing a crucial role in tolerance and the immune response to cancer. These findings strengthen the notion that pancreatic cancers induce antitumor immune responses. Therefore, attempts to improve the clinical efficacy of immunotherapy should involve strategies to neutralize or overcome immune suppression.

12.3 Cellular Targets in Pancreatic Cancer

The expression of an antigen – either mutated or unaltered self – must be restricted to the tumor or only minimally expressed elsewhere in the body to be considered an ideal tumor vaccine candidate. Table 12.1 enumerates a limited list of tumor antigens that fulfill this criterion for pancreatic cancer.

12.4 Immunotherapies in Pancreatic Cancer

Both active and passive immunity have been tested in pancreatic cancer (PC) to elicit immune responses to tumor cells. Targeting active immunity through vaccines attempts to induce long-term cellular (T-cell) immunity against cancer cells, whereas antibody-based immunotherapy targets PC cells, but does not stimulate long-term immunity. Recent active and passive immunotherapies in PC will be discussed in this section.

Table 12.1 Candidate pancreas cancer-associated antigens for immune targeting [29–52]

Antigen	Location	Expression in tumor	Prevalence (%)	Description
CEA	Cell surface (GPI-linked)	Overexpressed	30–100	Glycoprotein, normally expressed only on oncofetal tissues. Functions as cell-adhesion molecule. First tumor antigen to be described
Her2-neu	Transmembrane	Overexpressed	>50	A receptor tyrosine kinase, member of the EGF–receptor family, involved in cell growth and differentiation
K-Ras	Intracellular	Mutated self	90	Mutated form of ras, a GTPase important for cell proliferation, differentiation, and survival
Mesothelin	Cell surface (GPI-linked)	Overexpressed	~100	GPI-linked glycoprotein normally expressed on the surface of mesothelial cells lining the pleura, peritoneum, and pericardium at low levels. Binding partner of CA125/MUC16
MUC-1	Transmembrane	Overexpressed, hypoglycosylation	90	Type 1 transmembrane glycoprotein, expressed on apical surface of ductal and glandular epithelial cells at low levels. Extracellular domain has a polypeptide core with multiple tandem repeats of 20 amino acids
p53	Intracellular	Mutated self	50–70	Tumor suppressor that regulates cell cycle. Normally inhibits survivin at the transcription level and can initiate apoptosis if DNA damage is unrepairable
Survivin	Intracellular	Overexpressed	80	Member of IAP family. Inhibits caspase activation; is found in most human tumors and fetal tissue, but is completely absent in terminally differentiated cells
Telomerase	Intracellular	Overexpressed	95	Ribonucleoprotein that is responsible for RNA-dependent synthesis of telomeric DNA. TERT is its catalytic subunit
VEGFR2	Transmembrane	Overexpressed	64	A tyrosine kinase and member of platelet-derived growth factor family. Receptor for VEGF with functions in blood vessel development

CEA carcinoembryonic antigen, *GPI* glycosylphosphatidylinositol, *IAP* inhibitor of apoptosis protein, *MUC* mucin, *TERT* telomerase reverse transcriptase, *VEGFR* VEGF receptor

12.4.1 Active Immunotherapy

The goal of tumor-specific vaccines is to present tumor-associated antigens (TAA) to immune cells and produce potent and lasting cytotoxic effects against tumor cells. Antigen presenting cells (APC) such as dendritic cells (DCs) and T cells (CD4/CD8) are the targets and effectors of this immune response. Different types of vaccines have been developed and specific examples are reviewed below.

12.4.1.1 Whole Cell Vaccines

Using irradiated tumor cell vaccines can produce potent immune responses to multiple TAAs present on pancreatic tumor cells. Allogeneic or autologous tumor cells can be used to develop vaccines. Advantages of whole cell vaccines include tumor cells can be grown *in vitro*, specific TAAs do not need to be identified, polyclonal tumor specific T cell populations are generated, and cells can be altered to express surface proteins or secretable factors that induce strong immune responses [53].

One such example is granulocyte-macrophage colony stimulating factor (GM-CSF) secreting tumor cells. Dranoff has previously shown that GM-CSF secreting cells induce long-lasting immunity in melanoma models [54]. A phase I study by Jaffee et al. took 14 patients

with PC and inoculated variable doses of GM-CSF secreting allogeneic tumor cells 8 weeks after pancreaticoduodenectomy. Three patients developed delayed-type hypersensitivity responses and remained disease free at 25 months [55].

A phase II, single-institution study treated 60 patients after surgical resection (R0 or R1) with 5×10^8 GM-CSF secreting tumor cells 8–10 weeks after surgery [56]. Patients then received 5 FU chemoradiotherapy and up to four more vaccinations. Median disease-free survival and overall survival were 17.3 and 24.8 months, respectively. The most common side effects were erythema, induration and pain at the vaccination site, and the only grade 3–4 side effect was eosinophilia in two patients (1 %). Disease-free survival was correlated with the induction of mesothelin-specific $CD8^+$ T cells. Mesothelin is a membrane-bound protein that is highly expressed in pancreatic cancer cells, but has low expression in normal tissue, making it a possible target of immune therapy [39] Laheru and colleagues showed that in metastatic cancer, a GM-CSF tumor cell vaccine given with cyclophosphamide also induces a mesothelin-specific $CD8^+$ T-cell response [57].

Cell surface molecule expression can be modified to induce immune responses. The most successful model exploits hyperacute rejection due to alpha-galactosyl (αGal). Nonprimate mammals express αGal, but humans have a nonfunctional gene. Repeated exposure by gut flora to αGal epitopes leads to high expression of anti-αGal antibodies, which constitutes up to 1 % of circulating IgG [58]. The hyperacute antibody-mediated rejection leads to cell-mediated immunity against TAA in murine models of melanoma [59]. A phase II open-label multi-institutional trial evaluated algenpantucel-L with adjuvant gemcitabine and 5-FU-based radiotherapy after surgical resection [60]. Adjuvant therapy was the same as the RTOG-9704 study protocol [61]; 73 patients were enrolled and 69 received 100 million or 300 million cells per injection and a median of 12 vaccinations. The primary endpoint of 1-year disease-free survival was 62 % and secondary endpoint of 1-year overall survival was 86 % with no serious side effects attributed to immunotherapy. Survival with the addition of algenpantucel-L compared favorably with RTOG-9704 study. A phase III multicenter randomized controlled trial to evaluate adjuvant gemcitabine alone or with 5-FU-based radiotherapy with or without algenpantucel-L was completed in January 2014 [62].

12.4.1.2 Peptide Vaccines

Small antigenic protein fragments are used to develop peptide vaccines. These peptides can be produced economically and safely with no risk of infectious material. In addition, no autologous tissue is required. The drawbacks are that they can be poorly immunogenic and adjuvants may be required to induce a meaningful response [63]. Multiple peptide vaccines have been developed to target PC and have shown promising results.

12.4.1.3 KRAS Vaccines

The v-Ki-ras2 Kristin rat sarcoma (KRAS) viral oncogene encodes a GTPase important for signal transduction. Mutations can be found in the majority of pancreatic cancers as well as in lung and colon cancers [36] In 1995, Gjertsen showed that T-cell response could be activated using a Kristin rat sarcoma mutant (codon 12) peptide vaccine [64]. A follow-up phase I/II study exposed autologous APCs to Kristin rat sarcoma peptide vaccine *ex vivo*, then reinfused the activated APCs in five patients. Two out of five produced immune responses to ras [65]. To improve immunogenicity of the vaccine, GM-CSF was used as an adjuvant to the K-Ras vaccine in 48 patients with PC. The vaccine was well tolerated and patients who showed an immune response had superior survival compared to nonresponders (148 days *vs.* 61 days) [66]. Unfortunately, a follow-up study showed the safety of k-Ras/GM-CSF vaccine, but did not produce an immune response [67].

Weden and colleagues were able to induce immune responses using Kristin rat sarcoma vaccines prepared with long synthetic peptides. These peptides require processing and presentation by APC and induce polyclonal T cells that have specificity to mutated Kristin rat sarcoma [68]. In 23 patients who were vaccinated after surgical resection (20 evaluable), 4/20 (20 %)

were alive at 10 years, whereas 0/87 in a cohort of nonvaccinated patients during that same period were alive [69].

12.4.1.4 VEGF Vaccines

Vascular endothelial growth factor (VEGF) is a key signaling protein in angiogenesis. Overexpression of VEGF is seen in pancreatic cancers and is associated with larger tumor size and enhanced local spread [52]. A phase I study used VEGFR2-169 epitope, a peptide vaccine for VEGF receptor 2, in combination with gemcitabine in advanced PC (unresectable or metastatic disease). Of the 18 patients receiving at least 1 vaccination, 61 % developed cytotoxic T lymphocytes specific to VEGFR2 and median survival was 8.7 months [70]. Recently, the results of a phase II/III randomized placebo controlled study of gemcitabine +/−VEGFR2 vaccine showed no survival advantage [71].

12.4.1.5 Telomerase Vaccines

Telomeres are nucleotide sequence repeats at the ends of chromatid that help maintain chromosomes. Telomerase helps to maintain the telomeres, and is reactivated in 85 % of pancreatic cancers [72]. A phase I/II trial looking at GV1001, a peptide vaccine based on the catalytic subunit of telomerase was tested in 48 patients with unresectable pancreatic cancer who were injected with one of three dose levels. The intermediate dose group (300 nmol) showed a median survival of 8.6 months, significantly higher than other groups, and a 1-year survival of 25 % [73]. Follow-up phase III trial did not show a survival advantage of GV1001 versus gemcitabine, but other phase III trials evaluating combination therapy are ongoing [74].

12.4.1.6 Recombinant Vaccines

To increase antigenicity, viral and bacterial antigens can be added to cancer vaccines to induce a more potent immune response. These infectious antigens can activate the innate immune system, thereby recruiting APC to the site [72]. One example is the TRICOM vaccine [75]. This poxvirus-based vaccination uses B7-1, ICAM-1, and LFA-3 to enhance T-cell stimulation. Kaufman combined CEA and MUC1 antigens with TRICOM expressing vaccinia (PANVAC-V) or fowlpox (PANVAC-F) in a phase I trial. Ten patients with advanced PC were primed with PANVAC-V, and then given three boosters of PANVAC-F monthly up to 12 months. Antibodies to vaccinia were seen in all ten patients and five of eight evaluable patients developed antigen-specific T-cell responses. Median overall survival was 6.3 months and significant prolonged survival was seen in patients who developed anti-CEA and anti-MUC1 immune responses (15.1 months versus 3.9 months) [76].

Listeria vaccines have also been combined with TAA in cancer vaccines. A trial of 28 patients looked at patients with hepatic metastases from four primary tumors (pancreatic, mesothelioma, ovarian, and nonsmall-cell lung cancer). Patients were administered live attenuated listeria vaccine expressing mesothelin, which is expressed on the cell surface of the tumors. An overall of 37 % of subjects lived >15 months with minimal adverse events; half of these patients had PC [77].

12.4.1.7 DNA Vaccines

Vaccines using DNA encoding TAAs have also shown some efficacy in murine models of PC. MUC-1 DNA vaccine was injected in mice along with pancreatic cancer cells expressing MUC-1 (panc02-MUC1) or no MUC-1 (panc02). Vaccinated mice developed cytotoxic T-cell responses to MUC-1 and tumor shrinkage and improved survival was seen in mice with panc02-MUC1 cells. Mice injected with panc02 cells did not show any therapeutic benefit [78].

Survivin has also been tested as a DNA vaccine. A member of the inhibitor apoptosis family, survivin expression is found in PC cells, but not normal pancreatic tissue [48]. Zhu and colleagues inoculated mice with a survivin DNA vaccine or control vector followed by panc02 cells. Survivin inoculated mice showed increased lymphocyte infiltration of tumors compared with control mice. Increased survival and decreased tumor size was also seen in the Survivin group [79]. These findings need to be tested further in human subjects.

12.4.1.8 Antigen-Pulsed Dendritic Cells

Antigen presenting cells (APC) acquire and process antigens to present to T-cells on MHC class I and II molecules. Dendritic cells (DC) are the most important APC, often referred to as "professional APC." Multiple DC vaccines have been developed and sipuleucel-T is known as the most successful that improved overall survival in castration-resistant prostate cancer versus placebo in a phase III trial [80]. Multiple DC vaccines have been tested in pancreatic cancer with encouraging results. A phase I/II study took autologous DC pulsed with MUC1 peptide and treated 12 patients with resected pancreatic and biliary tumors. No significant toxicity was seen and four patients were alive at 4 years [81]. MUC1-pulsed DC have also been tested in advanced stage PC in a recent pilot study [82]. The vaccine was well tolerated in all seven enrolled patients with no significant side effects. This phase I study did not show a clinical benefit, but confirmed the safety of the vaccine and needs further study.

Another study looked at DC pulsed with carcinoembryonic antigen (CEA) mRNA. Patients with resected PC following neoadjuvant chemoradiotherapy were given autologous DC vaccine for 6 months. All three patients treated were alive 2.5 years after diagnosis with no evidence of disease [83].

12.4.2 Passive Immunotherapy

12.4.2.1 Antibody-Based Therapies

Monoclonal antibodies (mAbs) can affect tumor cells by a number of different mechanisms. Cytotoxic effects including Antibody-dependent cell-mediated cytotoxicity (ADCC), complement-mediated cytotoxicity (CMC), and antibody-dependent cellular phagocytosis leading to apoptosis as well as blockade of cellular receptors-growth factor/cytokine interaction inhibiting growth and survival can be exploited by antibody therapy [84]. Immunoglobulins can also be conjugated to radioisotopes or cytotoxic agents (chemotherapeutic agents, or toxins) and target-specific cellular targets to limit side effects. Numerous studies have evaluated the role of mAb in pancreatic cancer.

Various proteins expressed by PC cells have been targeted by mAb. One such protein expressed by multiple cancer cells is mesothelin. The vast majority of adenocarcinoma of the pancreas express mesothelin, but it is not seen in normal pancreatic tissue or chronic pancreatitis [38]. A phase I study involving 24 patients with mesothelioma, ovarian and pancreatic cancer testing an mAb to mesothelin (MORAb-009) was reported in 2010. MORAb-009 was well tolerated at 200 mg/m^2 weekly and 11 patients showed stable disease. One pancreatic cancer patient who progressed on gemcitabine had stable disease for 6 months [85]. A recent phase II study looking at MORAb-009 with gemcitabine versus gemcitabine alone has now been completed and results are pending [112].

Epidermal growth factor receptor (EGFR) is a glycoprotein receptor key in signaling cell proliferation and has been a successful target in numerous cancers including lung, head, and neck cancers. Multiple trials have tested addition of EGFR inhibition to standard chemotherapy in pancreatic cancer. Erlotinib, a small molecule tyrosine kinase inhibitor of EGFR has shown improved survival, but immunotherapy trials have not been as successful [86]. Cetuximab, a chimeric mAb was tested in a large phase III trial run by SWOG comparing gemcitabine with cetuximab versus gemcitabine alone in advanced pancreatic cancer patients. No improvement in progression-free or overall survival was seen with the addition of cetuximab [87]. A fully humanized antibody to EGFR, matuzumab, has also been tested in pancreatic cancer. A phase I study combining matuzumab with gemcitabine was well tolerated and 67 % (8/12 patients) showed partial response or stable disease [88].

Trastuzumab is an mAb that binds epidermal growth factor receptor 2 (HER2), a tyrosine kinase receptor that is part of the EGFR family. It has been effective in both breast and gastric cancers, and is expressed in a significant percent of pancreatic tumors [53]. Treatment with trastuzumab in mouse models has shown efficacy.

Nude mice injected with human pancreatic tumor cells showed prolonged survival and decreased metastasis when treated with trastuzumab [89]. Combination therapy has also shown benefit. Larbouret showed that trastuzumab combined with cetuximab resulted in superior survival in mice with human pancreatic cancer xenografts compared to gemcitabine [90]. A phase I/II trial of trastuzumab and cetuximab as second-line therapy in metastatic PC has been completed, but results are not published [91].

Angiogenesis has also been a target of passive immunotherapy, specifically antibodies to vascular endothelial growth factor (VEGF). As noted previously, VEGF and its receptors are often overexpressed in PC [52], and a phase II study showed partial response (21 %) and stable disease (46 %) in metastatic PC patients treated with bevacizumab (anti-VEGF Ab) and gemcitabine [92]. Benefit was not seen in the phase III follow-up study by the Cancer and Leukemia group B (CALGB80303). Overall, 602 patients with advanced PC (85 % metastatic disease) were randomly assigned to gemcitabine+bevacizumab or gemcitabine+placebo. No statistical difference in progression-free or overall survival was seen with the addition of bevacizumab [93]. Bevacizumab has also been tested in combination with gemcitabine and erlotinib in the AVITA study. The addition of bevacizumab increased progression-free survival, but not overall survival [94].

12.5 Radioimmunotherapy

Delivery of radioactive substances by tumor-specific antibodies has produced promising results. Anti-Muc-1 antibody, PAM4, is expressed in 85 % of pancreatic cancers, but not in normal pancreatic tissue [95]. Humanized PAM4 (clivatuzumab tetraxetan) conjugated with yttrium-90, a beta-emitting nucleotide with a radiation path length of 5 mm (^{90}Y-hPAM4), was studied in a phase I trial of 38 untreated patients with advanced PC (86 % stage IV disease). Weekly gemcitabine was used as a radiosensitizer and ^{90}Y-hPAM4 was given weekly starting week 2 on 4 week cycles. Six patients had partial responses (16 %) and 16 (42 % showed stable disease). Median survival was 7.7 months. Grade 3–4 thrombocytopenia or neutropenia developed in 20/38 treated patients after cycle 1. The authors concluded that ^{90}Y-hPAM4 has promising therapeutic activity with manageable side effects [96].

12.6 Immunoconjugates

Antibodies conjugated to cytotoxic agents can concentrate chemotherapy or other toxins at tumor sites and spare normal tissue. CEA has been the target of early immunoconjugate studies in PC. CEA is frequently overexpressed in gastrointestinal tumors including PC. hMN-14 or labetuzumab is an anti-CEA antibody that induces ADCC *in vitro* in murine colon cancer models [97]. In a pilot study, Labetuzumab was conjugated to SN-38 and infused to mice with human colon and pancreatic cancer xenografts. SN-38 is the active metabolite (two to three times potency) of irinotecan. Improved survival and decreased tumor size was observed in both colon and pancreatic cancer xenografts compared to controls [98].

12.7 Pancreatic Neuroendocrine Immunotherapy

Pancreatic neuroendocrine (pNET) or islet cell tumors comprise 2–3 % of primary pancreatic tumors with increasing incidence over the past 30 years [99, 100]. Early-stage disease is treated with surgical resection, but until recently few options were available for metastatic or unresectable disease. Cytotoxic agents such as streptozocin, dacarbazine, and temozolomide have shown activity, but their application has been limited due to side effects.

Immunotherapy has also been previously studied with interferon. Antitumor effects with interferon alpha include T-cell stimulation as well as cell cycle arrest [101]. Retrospective studies have reported improvement in symptoms and tumor stabilization, but two prospective trials comparing somatostatin analogs with or without

interferon therapy did not show any significant difference in tumor response rates or progression-free survival [102, 103]. A large multicenter trial evaluating octreotide with either interferon or bevacizumab is currently enrolling [104].

New targeted therapies including everolimus (mTOR inhibitor) and sunitinib, along with octreotide have shown improvement in progression-free survival [105–107]. Sunitinib is a multikinase inhibitor that binds to various tyrosine kinase receptors including VEGF receptor. VEGF expression has been associated with risk of metastasis in low-grade neuroendocrine tumors [108]. Anti-VEGF antibody bevacizumab has shown activity in combination with gemcitabine and S1 in PC mouse models [109]. A phase II study by Chan and colleagues looked at 34 patients with gastroenteropancreatic neuroendocrine tumors (44 % pNET, 56 % carcinoid) treated with bevacizumab plus temozolomide. Overall response rate was 15 %, but 33 % of pNET patients (5/15) had a response compared to 0/19 with carcinoid tumors. Both median progression-free (14.3 months *vs.* 7.3 months) and overall survival (41.7 months vs. 18.8 months) were higher in pNET versus carcinoid [110].

Little research has been pursued in vaccine development for pancreatic neuroendocrine tumors. A case report using autologous DCs pulsed with tumor cell lysate was delivered subcutaneously to a patient with metastatic pNET. A DTH reaction developed and specific T-cell response was noted. The patient had stable disease at 20 months after starting therapy [111].

12.8 Concluding Remarks

Pancreatic cancer continues to be a highly lethal disease in which new therapeutics are desperately needed. Immunotherapy in pancreatic cancer is in its infancy, but gives new targets and new ways to deliver therapeutics to the tumor. Vaccines, mAbs and drug- or radioimmunotherapy has shown promising results in multiple preclinical models and numerous therapies are currently in clinical trials. Although no current immunotherapy is standard of care in pancreatic cancers, they may prove to be key components of treatment in the future.

References

1. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin. 2010;60:277–300.
2. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61:69–90.
3. Oettle H, Post S, Neuhaus P, et al. Adjuvant chemotherapy with gemcitabine vs observation in patients undergoing curative-intent resection of pancreatic cancer: a randomized controlled trial. JAMA. 2007;297:267–77.
4. Conroy T, Desseigne F, Ychou M, et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. N Engl J Med. 2011;364:1817–25.
5. Goldman B, DeFrancesco L. The cancer vaccine roller coaster. Nat Biotechnol. 2009;27(2):129–39.
6. Yokokawa J, Palena C, Arlen P, et al. Identification of novel human CTL epitopes and their agonist epitopes of mesothelin. Clin Cancer Res. 2005;11(17):6342–51.
7. Andersen MH, Pedersen LO, Becker JC, Straten PT. Identification of a cytotoxic T lymphocyte response to the apoptosis inhibitor protein survivin in cancer patients. Cancer Res. 2001;61(3):869–72.
8. Johnston FM, Tan MC, Tan Jr BR, et al. Circulating mesothelin protein and cellular antimesothelin immunity in patients with pancreatic cancer. Clin Cancer Res. 2009;15(21):6511–18.
9. Kotera Y, Fontenot JD, Pecher G, Metzgar RS, Finn OJ. Humoral immunity against a tandem repeat epitope of human mucin MUC-1 in sera from breast, pancreatic, and colon cancer patients. Cancer Res. 1994;54(11):2856–60.
10. Kubuschok B, Neumann F, Breit R, et al. Naturally occurring T-cell response against mutated p21 ras oncoprotein in pancreatic cancer. Clin Cancer Res. 2006;12(4):1365–72.
11. Wenandy L, Sorensen RB, Sengelov L, Svane IM, Thor SP, Andersen MH. The immunogenicity of the hTERT540–548 peptide in cancer. Clin Cancer Res. 2008;14(1):4–7.
12. Yanagimoto H, Mine T, Yamamoto K, et al. Immunological evaluation of personalized peptide vaccination with gemcitabine for pancreatic cancer. Cancer Sci. 2007;98(4):605–11.
13. Dunn GP, Koebel CM, Schreiber RD. Interferons, immunity and cancer immunoediting. Nat Rev Immunol. 2006;6(11):836–48.
14. Swann JB, Smyth MJ. Immune surveillance of tumors. J Clin Invest. 2007;117(5):1137–46.
15. Hamanaka Y, Suehiro Y, Fukui M, Shikichi K, Imai K, Hinoda Y. Circulating anti-MUC1 IgG antibodies as a favorable prognostic factor for pancreatic cancer. Int J Cancer. 2003;103(1):97–100.
16. Pages F, Galon J, Dieu-Nosjean MC, Tartour E, Sautes-Fridman C, Fridman WH. Immune infiltration in human tumors: a prognostic factor that should not be ignored. Oncogene. 2010;29(8):1093–102.

17. Clark CE, Beatty GL, Vonderheide RH. Immunosurveillance of pancreatic adenocarcinoma: insights from genetically engineered mouse models of cancer. Cancer Lett. 2009;279(1):1–7.
18. Fong L, Small EJ. Anti-cytotoxic T-lymphocyte antigen-4 antibody: the first in an emerging class of immunomodulatory antibodies for cancer treatment. J Clin Oncol. 2008;26(32):5275–83.
19. Paulos CM, June CH. Putting the brakes on BTLA in T cell-mediated cancer immunotherapy. J Clin Invest. 2010;120(1):76–80.
20. Pardoll D. Does the immune system see tumors as foreign or self? Annu Rev Immunol. 2003;21:807–39.
21. Ikemoto T, Yamaguchi T, Morine Y, et al. Clinical roles of increased populations of $Foxp3^{+}CD4^{+}$ T cells in peripheral blood from advanced pancreatic cancer patients. Pancreas. 2006;33(4):386–90.
22. Hiraoka N, Onozato K, Kosuge T, Hirohashi S. Prevalence of $FOXP3^{+}$ regulatory T cells increases during the progression of pancreatic ductal adenocarcinoma and its premalignant lesions. Clin Cancer Res. 2006;12(18):5423–34.
23. Nomi T, Sho M, Akahori T, et al. Clinical significance and therapeutic potential of the programmed death-1 ligand/programmed death-1 pathway in human pancreatic cancer. Clin Cancer Res. 2007;13(7):2151–57.
24. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science. 2003;299(5609):1057–61.
25. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol. 1995;155(3):1151–64.
26. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of $CD4^{+}CD25^{+}$ regulatory T cells. Nat Immunol. 2003;4(4):330–36.
27. Khattri R, Cox T, Yasayko SA, Ramsdell F. An essential role for Scurfin in $CD4^{+}CD25^{+}$ T regulatory cells. Nat Immunol. 2003;4(4):337–42.
28. Linehan DC, Goedegebuure PS. $CD25^{+}$ $CD4^{+}$ regulatory T-cells in cancer. Immunol Res. 2005;32(1–3):155–16.
29. Huang EH, Kaufman HL. CEA-based vaccines. Expert Rev Vaccines. 2002;1(1):49–63.
30. Beatty JD, Romero C, Brown PW, Lawrence Jr W, Terz JJ. Clinical value of carcinoembryonic antigen: diagnosis, prognosis, and follow-up of patients with cancer. Arch Surg. 1979;114(5):563–67.
31. Ona FV, Zamcheck N, Dhar P, Moore T, Kupchik HZ. Carcinoembryonic antigen (CEA) in the diagnosis of pancreatic cancer. Cancer. 1973;31(2):324–27.
32. Ladjemi MZ, Jacot W, Chardes T, Pelegrin A, Navarro-Teulon I. Anti-HER2 vaccines: new prospects for breast cancer therapy. Cancer Immunol Immunother. 2010;59(9):1295–12.
33. Lei S, Appert HE, Nakata B, Domenico DR, Kim K, Howard JM. Overexpression of HER2/neu oncogene in pancreatic cancer correlates with shortened survival. Int J Pancreatol. 1995;17(1):15–21.
34. Yamanaka Y, Friess H, Kobrin MS, et al. Overexpression of *HER2/neu* oncogene in human pancreatic carcinoma. Hum Pathol. 1993;24(10):1127–34.
35. Downward J. Targeting RAS and PI3K in lung cancer. Nat Med. 2008;14(12):1315–16.
36. Almoguera C, Shibata D, Forrester K, Martin J, Arnheim N, Perucho M. Most human carcinomas of the exocrine pancreas contain mutant *c-K-ras* genes. Cell. 1988;53(4):549–54.
37. Li M, Bharadwaj U, Zhang R, et al. Mesothelin is a malignant factor and therapeutic vaccine target for pancreatic cancer. Mol Cancer Ther. 2008;7(2):286–96.
38. Hassan R, Laszik ZG, Lerner M, Raffeld M, Postier R, Brackett D. Mesothelin is overexpressed in pancreaticobiliary adenocarcinomas but not in normal pancreas and chronic pancreatitis. Am J Clin Pathol. 2005;124(6):838–45.
39. Argani P, Iacobuzio-Donahue C, Ryu B, et al. Mesothelin is overexpressed in the vast majority of ductal adenocarcinomas of the pancreas: identification of a new pancreatic cancer marker by serial analysis of gene expression (SAGE). Clin Cancer Res. 2001;7(12):3862–68.
40. Kaneko O, Gong L, Zhang J, et al. A binding domain on mesothelin for CA125/MUC16. J Biol Chem. 2009;284(6):3739–49.
41. Tang CK, Katsara M, Apostolopoulos V. Strategies used for MUC1 immunotherapy: human clinical studies. Expert Rev Vaccines. 2008;7(7):963–75.
42. Qu CF, Li Y, Song YJ, et al. MUC1 expression in primary and metastatic pancreatic cancer cells for *in vitro* treatment by (213)Bi-C595 radioimmunoconjugate. Br J Cancer. 2004;91(12):2086–93.
43. Chen F, Wang W, El-Deiry WS. Current strategies to target p53 in cancer. Biochem Pharmacol. 2010;80(5):724–30.
44. Scarpa A, Capelli P, Mukai K, et al. Pancreatic adenocarcinomas frequently show *p53* gene mutations. Am J Pathol. 1993;142(5):1534–43.
45. Ryan BM, O'Donovan N, Duffy MJ. Survivin: a new target for anti-cancer therapy. Cancer Treat Rev. 2009;35(7):553–62.
46. Kanwar RK, Cheung CH, Chang JY, Kanwar JR. Recent advances in anti-survivin treatments for cancer. Curr Med Chem. 2010;17(15):1509–15.
47. Qiao JG, Zhang YQ, Yin YC, Tan Z. Expression of Survivin in pancreatic cancer and its correlation to expression of Bcl-2. World J Gastroenterol. 2004;10(18):2759–61.
48. Satoh K, Kaneko K, Hirota M, Masamune A, Satoh A, Shimosegawa T. Expression of survivin is correlated with cancer cell apoptosis and is involved in the development of human pancreatic duct cell tumors. Cancer. 2001;92(2):271–8.
49. Liu JP, Chen W, Schwarer AP, Li H. Telomerase in cancer immunotherapy. Biochim Biophys Acta. 2010;1805(1):35–42.

50. Hiyama E, Kodama T, Shinbara K, et al. Telomerase activity is detected in pancreatic cancer but not in benign tumors. Cancer Res. 1997;57(2):326–31.
51. Shibuya M. Vascular endothelial growth factor (VEGF)-receptor2: its biological functions, major signaling pathway, and specific ligand VEGF-E. Endothelium. 2006;13(2):63–9.
52. Itakura J, Ishiwata T, Friess H, et al. Enhanced expression of vascular endothelial growth factor in human pancreatic cancer correlates with local disease progression. Clin Cancer Res. 1997;3(8):1309–16.
53. Niccolai E, Prisco D, D'Elios MM, Amedei A. What is recent in pancreatic cancer immunotherapy? BioMed Res Int. 2013;2013:492372.
54. Dranoff G, Jaffee EM, Lazenby A, et al. Vaccination with irradiated tumor cells engineered to secrete murine granulocyte/macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. Proc Natl Acad Sci U S A. 1993;90:3539–43.
55. Jaffee EM, et al. Novel allogeneic granulocyte-macrophage colony-stimulating factor-secreting tumor vaccine for pancreatic cancer: a phase I trial of safety and immune activation. J Clin Oncol. 2001;19(1):145–56.
56. Lutz E, et al. A lethally irradiated allogeneic granulocyte-macrophage colony stimulating factor-secreting tumor vaccine for pancreatic adenocarcinoma: a phase II trial of safety, efficacy, and immune activation. Ann Surg. 2011;253(2):328–35.
57. Laheru D, et al. Allogeneic granulocyte macrophage colony-stimulating factor-secreting tumor immunotherapy alone or in sequence with cyclophosphamide for metastatic pancreatic cancer: a pilot study of safety, feasibility, and immune activation. Clin Cancer Res. 2008;14(5):1455–63.
58. Galili U, et al. Evolutionary relationship between the natural anti-Gal antibody and the Gal alpha 1—3Gal epitope in primates. Proc Natl Acad Sci U S A. 1987;84(5):1369–73.
59. Rossi GR, et al. Allogeneic melanoma vaccine expressing alphaGal epitopes induces antitumor immunity to autologous antigens in mice without signs of toxicity. J Immunother. 2008;31(6):545–54.
60. Hardacre J, et al. Addition of algenpantucel-L immunotherapy to standard adjuvant therapy for pancreatic cancer: a phase 2 study. J Gastrointest Surg. 2013;17(1):94–100.
61. Regine W, et al. Fluorouracil vs gemcitabine chemotherapy before and after fluorouracil-based chemoradiation following resection of pancreatic adenocarcinoma: a randomized controlled trial. JAMA. 2008;299(9):1019–26.
62. Clinicaltrials.gov identifier: NCT01072981. Principal director: Nicholas N Vahanian, M.D. A Phase III study of chemotherapy and chemoradiotherapy with or without hyperacute®-pancreas (algenpantucel-L) immunotherapy in subjects with surgically resected pancreatic cancer. Opened 2/18/2010.
63. Purcell A, et al. More than one reason to rethink the use of peptides in vaccine design. Nat Rev Drug Discov. 2007;6(5):404–14.
64. Gjertsen M. Vaccination with mutant ras peptides and induction of T-cell responsiveness in pancreatic carcinoma patients carrying the corresponding RAS mutation. Lancet. 1995;346(8987):1399–400.
65. Gjertsen M, et al. Ex vivo ras peptide vaccination in patients with advanced pancreatic cancer: results of a phase I/II study. Int J Cancer. 1996;65(4):450–3.
66. Gjertsen M, et al. Intradermal ras peptide vaccination with granulocyte-macrophage colony-stimulating factor as adjuvant: clinical and immunological responses in patients with pancreatic adenocarcinoma. Int J Cancer. 2001;92(3):441–50.
67. Abou-Alfa G, et al. Targeting mutated K-ras in pancreatic adenocarcinoma using an adjuvant vaccine. Am J Clin Oncol. 2011;34(3):321–5.
68. Bijker M, et al. Superior induction of anti-tumor CTL immunity by extended peptide vaccines involves prolonged, DC-focused antigen presentation. Eur J Immunol. 2008;38(4):1033–42.
69. Weden S, et al. Long-term follow-up of patients with resected pancreatic cancer following vaccination against mutant K-ras. Int J Cancer. 2011;128(5):1120–8.
70. Miyazawa M, et al. Phase I clinical trial using peptide vaccine for human vascular endothelial growth factor receptor 2 in combination with gemcitabine for patients with advanced pancreatic cancer. Cancer Sci. 2010;101(2):433–9.
71. Yamaue H, et al. Phase II/III clinical trial with VEGFR2-epitope peptide and gemcitabine for patients with locally advanced, metastatic, or unresectable pancreatic cancer: Pegasus-PC study. J Clin Oncol. 2012;30(Suppl 34):abstr 223.
72. Soares K, et al. Vaccines for pancreatic cancer. Cancer J. 2012;18(6):642–52.
73. Bernhardt S, et al. Telomerase peptide vaccination of patients with non-resectable pancreatic cancer: a dose escalating phase I/II study. Br J Cancer. 2006;95(11):1474–82.
74. Gunturu K, et al. Immunotherapy updates in pancreatic cancer: are we there yet? Ther AdvMed Oncol. 2013;5(1):81–9.
75. Garnett C, et al. TRICOM vector based cancer vaccines. Curr Pharm Des. 2006;12(3):351–61.
76. Kaufman H, et al. Poxvirus-based vaccine therapy for patients with advanced pancreatic cancer. J Transl Med. 2007;5:60.
77. Le D, et al. A live-attenuated Listeria vaccine (ANZ-100) and a live-attenuated Listeria vaccine expressing mesothelin (CRS-207) for advanced cancers: phase I studies of safety and immune induction. Clin Cancer Res. 2012;18(3):858–68.
78. Rong Y, et al. Induction of protective and therapeutic anti-pancreatic cancer immunity using a reconstructed MUC1 DNA vaccine. BMC Cancer. 2009;9:191.
79. Zhu K, et al. Survivin DNA vaccine generated specific antitumor effects in pancreatic carcinoma and

lymphoma mouse models. Vaccine. 2007;25(46): 7955–61.
80. Kantoff P, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med. 2010;363(5):411–22.
81. Lepisto A, et al. A phase I/II study of a MUC1 peptide pulsed autologous dendritic cell vaccine as adjuvant therapy in patients with resected pancreatic and biliary tumors. Cancer Ther. 2008;6(B):955–64.
82. Rong Y, et al. A phase I pilot trial of MUC1-peptide-pulsed dendritic cells in the treatment of advanced pancreatic cancer. Clin Exp Med. 2012;12(3):173–80.
83. Morse M, et al. The feasibility and safety of immunotherapy with dendritic cells loaded with CEA mRNA following neoadjuvant chemoradiotherapy and resection of pancreatic cancer. Int J Gastrointest Cancer. 2002;32(1):1–6.
84. Jurcic J, et al. Monoclonal antibody therapy of cancer. In: Kaufman H, Wolchok JD, editors. General principles of tumor immunology: basic and clinical applications of tumor immunology. Dordrecht: Springer; 2008. p. 321–42.
85. Hassan R, et al. Phase I clinical trial of the chimeric anti-mesothelin monoclonal antibody MORAb-009 in patients with mesothelin-expressing cancers. Clin Cancer Res. 2010;16(24):6132–8.
86. Moore M, 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. 2007;25(15):1960–6.
87. Philip P, et al. Phase III study comparing gemcitabine plus cetuximab versus gemcitabine in patients with advanced pancreatic adenocarcinoma: Southwest Oncology Group-directed intergroup trial S0205. J Clin Oncol. 2010;28(22):3605–10.
88. Graeven U, et al. Phase I study of the humanised anti-EGFR monoclonal antibodymatuzumab (EMD 72000) combined with gemcitabine in advanced pancreatic cancer. Br J Cancer. 2006;94(9):1293–9.
89. Pratesi G, Petrangolini G, Tortoreto M, et al. Antitumor efficacy of trastuzumab in nude mice orthotopically xenografted with human pancreatic tumor cells expressing low levels of HER-2/neu. J Immunother. 2008;31(6):537–44.
90. Larbouret C, Robert B, Bascoul-Mollevi C, et al. Combined cetuximab and trastuzumab are superior to gemcitabine in the treatment of human pancreatic carcinoma xenografts. Ann Oncol. 2010;21(1):98–103.
91. ClinicalTrials.gov Identifier:NCT00923299. Cetuximab and trastuzumab in treating patients with metastatic pancreatic cancer that progressed after previous treatment with gemcitabine primary investigator: Marc Ychou MD, PhD. Opened 6/17/2009.
92. Kindler HL, Friberg G, Singh DA, et al. Phase II trial of bevacizumab plus gemcitabine in patients with advanced pancreatic cancer. J Clin Oncol. 2005;23(31):8033–40.
93. Kindler HL, Niedzwiecki D, Hollis D, et al. Gemcitabine plus bevacizumab compared with gemcitabine plus placebo in patients with advanced pancreatic cancer: phase III trial of the Cancer and Leukemia Group B (CALGB 80303). J Clin Oncol. 2010;28(22):3617–22.
94. Van Cutsem E, Vervenne WL, Bennouna J, et al. Phase III trial of bevacizumab in combination with gemcitabine and erlotinib in patients with metastatic pancreatic cancer. J Clin Oncol. 2009;27(13):2231–7.
95. Gold DV, Karanjawala Z, Modrak DE, et al. PAM4-reactive MUC1 is a biomarker for early pancreatic adenocarcinoma. Clin Cancer Res. 2007;13(24):7380–7.
96. Ocean AJ, Pennington KL, Guarino MJ, et al. Fractionated radioimmunotherapy with (90) Y-clivatuzumab tetraxetan and low-dose gemcitabine is active in advanced pancreatic cancer: a phase 1 trial. Cancer. 2012;118(22):5497–506.
97. Blumenthal RD, Osorio L, Hayes MK, et al. Carcinoembryonic antigen antibody inhibits lung metastasis and augments chemotherapy in a human colonic carcinoma xenograft. Cancer Immunol Immunother. 2005;54(4):315–27.
98. Govindan SV, Cardillo TM, Moon SJ, et al. CEACAM5-targeted therapy of human colonic and pancreatic cancer xenografts with potent labetuzumab-SN-38 immunoconjugates. Clin Cancer Res. 2009;15(19):6052–61.
99. Fesinmeyer MD, Austin MA, Li CI, et al. Differences in survival by histologic type of pancreatic cancer. Cancer Epidemiol Biomarkers Prev. 2005;14(7):1766–73.
100. Yao JC, Hassan M, Phan A, et al. One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. J Clin Oncol. 2008;26(18):3063–72.
101. Detjen KM, Welzel M, Farwig K, et al. Molecular mechanism of interferon alfa-mediated growth inhibition in human neuroendocrine tumor cells. Gastroenterology. 2000;118(4):735–48.
102. Faiss S, Pape U-F, Böhmig M, et al. Prospective, randomized, multicenter trial on the antiproliferative effect of lanreotide, interferon alfa, and their combination for therapy of metastatic neuroendocrine gastroenteropancreatic tumors – The International Lanreotide and Interferon Alfa Study Group. J Clin Oncol. 2003;21(14):2689–696.
103. Arnold R, Rinke A, Klose KJ, et al. Octreotide versus octreotide plus interferon-alpha in endocrine gastroenteropancreatic tumors: a randomized trial. Clin Gastroenterol Hepatol. 2005;3(8):761–71.
104. ClinicalTrials.gov Identifier:NCT00569127. Octreotide acetate and recombinant interferon alfa-2b or bevacizumab in treating patients with metastatic or locally advanced, high-risk neuroendocrine tumor.
105. Yao JC, Shah MH, Ito T, et al. RAD001 in advanced neuroendocrine tumors, third trial (RADIANT-3) study group. Everolimus for advanced pancreatic

neuroendocrine tumors. N Engl J Med. 2011;364(6):514–23.
106. Raymond E, Dahan L, Raoul JL, et al. Sunitinib malate for the treatment of pancreatic neuroendocrine tumors. N Engl J Med. 2011;364(6):501–13.
107. Rinke A, Müller HH, Schade-Brittinger C, Klose KJ, PROMID Study Group, et al. Placebo-controlled, double-blind, prospective, randomized study on the effect of octreotide LAR in the control of tumor growth in patients with metastatic neuroendocrine midgut tumors: a report from the PROMID Study Group. J Clin Oncol. 2009;27(28):4656–63.
108. Zhang J, Jia Z, Li Q, Wang L, et al. Elevated expression of vascular endothelial growth factor correlates with increased angiogenesis and decreased progression-free survival among patients with low-grade neuroendocrine tumors. Cancer. 2007;109(8):1478–86.
109. Kasuya K, Nagakawa Y, Suzuki M, et al. Combination therapy of gemcitabine or oral S-1 with the anti-VEGF monoclonal antibody bevacizumab for pancreatic neuroendocrinecarcinoma. Exp Ther Med. 2012;3(4):599–602.
110. Chan JA, Stuart K, Earle CC, et al. Prospective study of bevacizumab plus temozolomide in patients with advanced neuroendocrine tumors. J Clin Oncol. 2012;30(24):2963–8.
111. Schott M, Feldkamp J, Lettmann M, et al. Dendritic cell immunotherapy in a neuroendocrine pancreas carcinoma. Clin Endocrinol (Oxf). 2007;55(2):271–7.
112. ClinicalTrials.gov Identifier:NCT00570713. A phase 2 randomized, placebo-controlled, double-blind study of the efficacy of MORAb-009 in combination with gemcitabine in patients with advanced pancreatic cancer. Opened 7 Dec 2007.
113. Von Hoff D, Ervin T, Arena FP, et al. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. N Engl J Med. 2013;369(18):1691–703.