VOLUME 31 · NUMBER 7 · MARCH 1 2013

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

# Randomized Phase III Multi-Institutional Study of TNFerade Biologic With Fluorouracil and Radiotherapy for Locally Advanced Pancreatic Cancer: Final Results

*Joseph M. Herman, Aaron T. Wild, Hao Wang, Phuoc T. Tran, Kenneth J. Chang, Gretchen E. Taylor, Ross C. Donehower, Timothy M. Pawlik, Mark A. Ziegler, Hongyan Cai, Dionne T. Savage, Marcia I. Canto, Jason Klapman, Tony Reid, Raj J. Shah, Sarah E. Hoffe, Alexander Rosemurgy, Christopher L. Wolfgang, and Daniel A. Laheru*

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Published online ahead of print at www.jco.org on January 22, 2013.

Supported by GenVec.

J.M.H. and A.T.W. contributed equally to this work.

Presented as a poster at the 48th Annual Meeting of the American Society of Clinical Oncology, Chicago, IL, June 1-5, 2012.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Clinical trial information: NCT00051467.

Corresponding author: Joseph M. Herman, MD, MSc, Department of Radiation Oncology & Molecular Radiation Sciences, Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins Hospital, 401 N. Broadway, Weinberg Suite 1440, Baltimore, MD 21231; e-mail: jherma15@jhmi.edu.



0732-183X/13/3107-886/$20.00

DOI: 10.1200/JCO.2012.44.7516

## ABSTRACT

### Purpose
TNFerade biologic is a novel means of delivering tumor necrosis factor alpha to tumor cells by gene transfer. We herein report final results of the largest randomized phase III trial performed to date among patients with locally advanced pancreatic cancer (LAPC) and the first to test gene transfer against this malignancy.

### Patients and Methods
In all, 304 patients were randomly assigned 2:1 to standard of care plus TNFerade (SOC + TNFerade) versus standard of care alone (SOC). SOC consisted of 50.4 Gy in 28 fractions with concurrent fluorouracil (200 mg/m$^2$ per day continuous infusion). TNFerade was injected intratumorally before the first fraction of radiotherapy each week at a dose of $4 \times 10^{11}$ particle units by using either a percutaneous transabdominal or an endoscopic ultrasound approach. Four weeks after chemoradiotherapy, patients began gemcitabine (1,000 mg/m$^2$ intravenously) with or without erlotinib (100 to 150 mg per day orally) until progression or toxicity.

### Results
The analysis included 187 patients randomly assigned to SOC + TNFerade and 90 to SOC by using a modified intention-to-treat approach. Median follow-up was 9.1 months (range, 0.1 to 50.5 months). Median survival was 10.0 months for patients in both the SOC + TNFerade and SOC arms (hazard ratio [HR], 0.90; 95% CI, 0.66 to 1.22; $P = .26$). Median progression-free survival (PFS) was 6.8 months for SOC + TNFerade versus 7.0 months for SOC (HR, 0.96; 95% CI, 0.69 to 1.32; $P = .51$). Among patients treated on the SOC + TNFerade arm, multivariate analysis showed that TNFerade injection by an endoscopic ultrasound-guided transgastric/transduodenal approach rather than a percutaneous transabdominal approach was a risk factor for inferior PFS (HR, 2.08; 95% CI, 1.06 to 4.06; $P = .032$). The patients in the SOC + TNFerade arm experienced more grade 1 to 2 fever and chills than those in the SOC arm ($P < .001$) but both arms had similar rates of grade 3 to 4 toxicities (all $P > .05$).

### Conclusion
SOC + TNFerade is safe but not effective for prolonging survival in patients with LAPC.

*J Clin Oncol 31:886-894. © 2013 by American Society of Clinical Oncology*

## INTRODUCTION

Pancreatic cancer is among the most lethal malignancies in the Western world, as attested to by a mortality that closely rivals its incidence.[1] Approximately 30% to 40% of patients present with unresectable, locally advanced pancreatic cancer (LAPC),[2,3] for which acceptable treatment options include chemoradiotherapy (CRT) or chemotherapy alone.[4-9] CRT typically results in tumor stability, with only a small subset of patients (10% to 15%) exhibiting an objective response.[10] Five-year survival for patients with LAPC remains dismal at less than 2%.[11] Novel treatments and methods of enhancing current therapeutic modalities are needed.

Tumor necrosis factor alpha is a potent inflammatory cytokine with substantial anticancer activity.[12-19] Multiple animal studies and clinical trials have shown that tumor necrosis factor alpha (TNF-$\alpha$) is effective against solid tumors, but the treatment has ultimately failed because of severe systemic toxicity consisting of hypotension and shock-like symptoms.[20-29] TNFerade biologic (GenVec, Gaithersburg, MD) represents a novel means of

selectively delivering TNF-$\alpha$ to tumor cells by gene transfer through intratumoral vector injection. TNFerade ($Ad_{GV}$EGR.TNF.11D) is a second generation E1-, E4-, and partial E3-deleted, replication-deficient adenovirus serotype 5 vector containing TNF-$\alpha$ cDNA ligated downstream from the early growth response protein 1 (Egr-1) promoter.[30] Egr-1 is induced by ionizing radiation,[31] thus allowing for spatial and temporal constraint of TNF-$\alpha$ production to the radiation field[17,30,32] and markedly attenuating systemic toxicity in preclinical studies.[33,34] Furthermore, spatiotemporal joining of irradiation and TNF-$\alpha$ production exploits documented synergy between these modalities.[21,33,35,36]

Early-phase clinical trials show encouraging evidence for local efficacy of TNFerade against multiple tumor types,[37,38] although TNFerade has not been observed to have any systemic anticancer activity. The predominant toxicities accompanying TNFerade administration in these trials were fever and chills. A phase I/II dose-escalation study in 50 patients with LAPC showed that TNFerade at doses of $4 \times 10^9$ to $4 \times 10^{11}$ particle units (PU) weekly with fluorouracil-based CRT was well-tolerated.[39] Furthermore, data were consistent with a dose-dependent increase in stabilization of treated tumors. On the basis of these results, a multicenter, randomized phase III trial of TNFerade in conjunction with CRT was conducted to assess efficacy and safety for LAPC.

## PATIENTS AND METHODS

### *Patients*

The study population consisted of patients with biopsy-confirmed, unresectable LAPC. Unresectable disease was defined by extension to the superior mesenteric artery and/or celiac axis with no fat plane separating the tumor and these arterial structures or obstruction of the superior mesenteric-portal

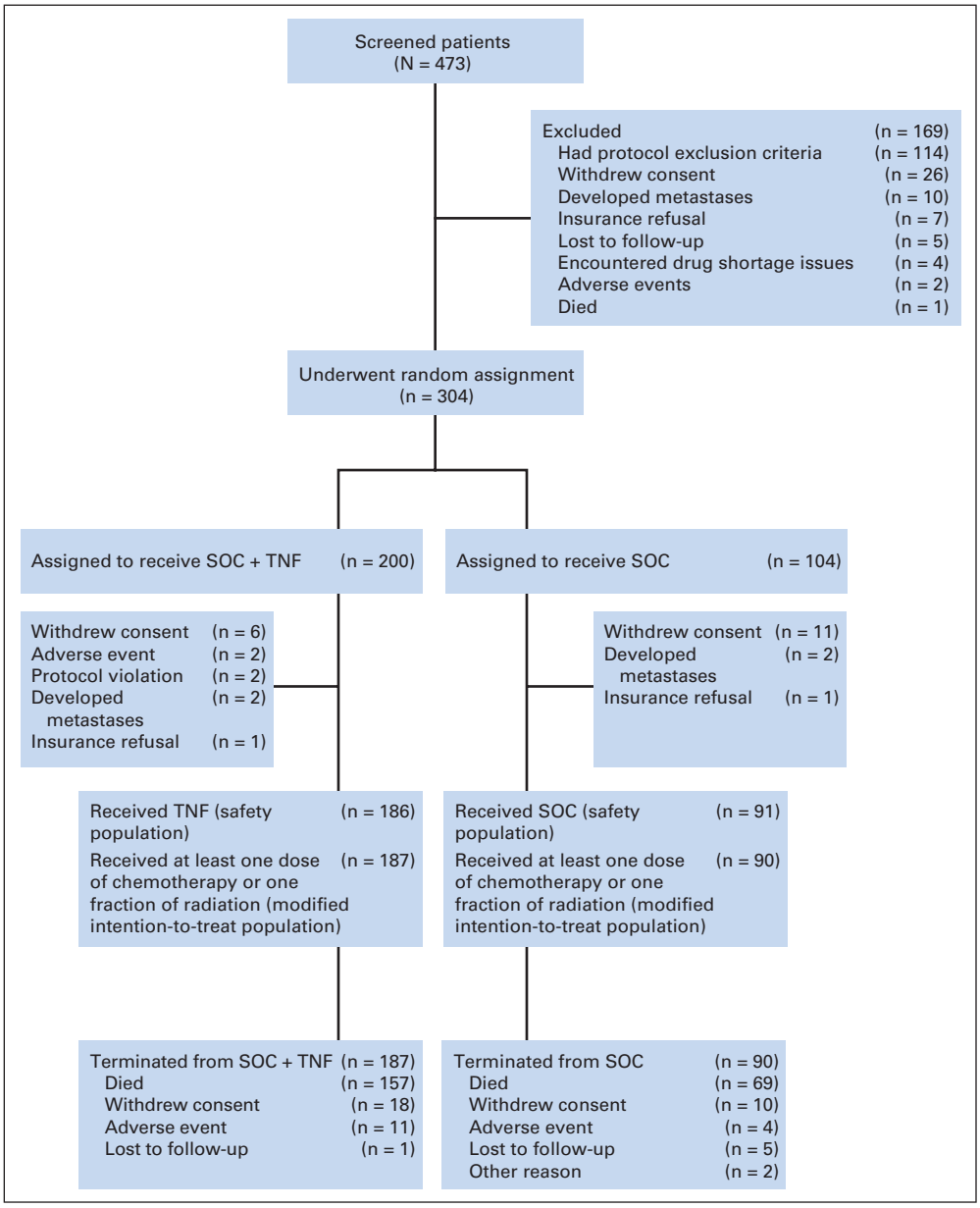


**Fig 1.** CONSORT diagram; enrollment and outcomes. SOC, standard of care; TNF, TNFerade.

vein confluence. Eligibility criteria included age ≥18 years, Karnofsky performance status (KPS) ≥ 70%,[40] life expectancy more than 3 months, and adequate hepatic, hematologic, immune, and renal function. Patients with technically resectable tumors (T1-T3) were also eligible if they were deemed unresectable because of medical comorbidities or refusal of surgery.

Exclusion criteria included evidence of metastatic disease, previous pancreatic cancer therapy, previous target field irradiation, clinically significant ascites, bulky celiac adenopathy (≥ 2.5 cm), or nonadenocarcinoma histology. It must be noted, however, that patients with unknown metastatic disease status (Mx) because of inability to obtain a contrast chest computed tomography (CT) scan or because of some other impediment to completing radiologic evaluation were allowed to enroll.

All patients provided written informed consent before enrollment. The study was approved by each center's institutional review board or ethics committee and complied with provisions of the Good Clinical Practice guidelines and Declaration of Helsinki, as well as with Food and Drug Administration regulations.

### Study Design

This open-label, randomized, controlled phase III trial was conducted at 39 sites throughout the continental United States. Eligible patients were randomly assigned 2:1 to the maximum-tolerated dose of TNFerade ($4 \times 10^{11}$ PU) plus standard-of-care therapy (SOC + TNFerade) versus standard-of-care therapy alone (SOC). Randomization was stratified by center and KPS (≥ 80% or < 80%). SOC consisted of continuous infusion fluorouracil and concurrent radiotherapy, followed by gemcitabine or gemcitabine plus erlotinib maintenance therapy at investigator discretion. TNFerade was administered by intratumoral injection by using a CT/ultrasound-guided percutaneous transabdominal approach (PTA) or an endoscopic ultrasound-guided (EUS) transgastric/transduodenal approach before radiotherapy on day 1 of each of the first 5 weeks of CRT. CRT took place on days 1 through 5 of each week. Radiotherapy consisted of 45 Gy delivered in 25 fractions of 1.80 Gy followed by a boost to the tumor plus a 1-cm margin consisting of 5.40 Gy in three fractions of 1.80 Gy. Radiotherapy was delivered by using three-dimensional conformal or intensity-modulated techniques. Concurrent fluorouracil was started before radiotherapy on day 1 of each week and administered by continuous infusion (200 mg/m$^2$ per day) until conclusion of radiotherapy each week. Four weeks following CRT, patients were allowed to begin maintenance gemcitabine (1,000 mg/m$^2$ intravenously on days 1, 8, and 15 of 4-week cycles) with/without erlotinib (100 to 150 mg per day orally). Maintenance therapy was continued until radiographically documented disease progression or unacceptable toxicity occurred. Excessive morbidity and mortality attributable to TNFerade was monitored by an independent data and safety monitoring board. Crossover between study arms was not permitted.

### Outcomes and Assessments

Overall survival was measured from date of random assignment until date of death. Predefined secondary outcomes included progression-free survival (PFS), tumor response rates, and surgical downstaging rates. An independent blinded central reading laboratory reviewed CT scans and magnetic resonance imaging (MRI) scans to assess for progression and tumor response according to Response Evaluation Criteria in Solid Tumors (RECIST).[41] Safety was assessed by using the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0 (NCI CTCAE v3.0).

### Statistical Analysis

The primary outcome was overall survival. A sample size of 299 patients (199 randomly assigned to SOC + TNFerade, 100 to SOC) was required to achieve 85% power to detect an absolute difference in overall survival rate at 12 months of 20% between the two treatment regimens, based on a two-sided $\chi^2$ test at a significance level of 0.025. The study planned to recruit 330 patients to account for 10% dropout. With this sample size, there would be 80% power to determine a hazard ratio (HR) of 0.667, assuming approximately 3.5 years of enrollment and 1 year of follow-up. Three interim analyses were planned, the first to assess for futility was based on response rate at 3 months, and the second and third interim analyses to test for superiority and futility were conducted after one third (92 deaths) and two thirds (184 deaths) of the total events required, respectively. The trial was discontinued following the third interim analysis on the basis of futility.

Analyses of primary and secondary efficacy end points of overall survival and PFS were based on a modified intention-to-treat population that included all randomly assigned patients who received at least one study treatment and considered allocation of patients to treatment groups as randomly assigned. Stratified log-rank tests and multivariate Cox regression were used to compare survival and progression between treatment groups. Multivariate Cox models included the following factors: treatment group, age, sex, KPS, cancer antigen 19-9 (CA19-9), T-stage, N-stage, M-stage, prior cancer history, prior cancer treatment, and study sites. Toxicities, adverse events (AEs), and compliance were compared by using Fisher's exact test. All tests were two-sided, and *P* values were not adjusted for multiple comparisons. Statistical analysis was performed with SAS version 9.3 (SAS Institute, Cary, NC).

**Table 1.** Baseline Characteristics of the Intention-to-Treat Population

| Characteristic | Total No. | Total % | FU/RT (SOC) No. | FU/RT (SOC) % | FU/RT + TNFerade No. | FU/RT + TNFerade % | *P* |
|---|---|---|---|---|---|---|---|
| No. of patients | 277 | | 90 | | 187 | | |
| Age, years | | | | | | | .85 |
| Mean | 63.5 | | 63.3 | | 63.5 | | |
| SD | 11.0 | | 10.7 | | 11.2 | | |
| Median | 64.4 | | 64.8 | | 64.3 | | |
| Range | 28.7-85.6 | | 28.7-84.8 | | 31.0-85.6 | | |
| Sex | | | | | | | .30 |
| Male | 160 | 57.8 | 48 | 53.3 | 112 | 59.9 | |
| Female | 117 | 42.2 | 42 | 46.7 | 75 | 40.1 | |
| Race | | | | | | | .72 |
| White | 212 | 76.5 | 72 | 80 | 140 | 74.9 | |
| African American | 34 | 12.3 | 11 | 12.2 | 23 | 12.3 | |
| Hispanic | 14 | 5.1 | 3 | 3.3 | 11 | 5.9 | |
| Other | 17 | 6.1 | 4 | 4.5 | 13 | 6.9 | |
| Prior cancer history | | | | | | | .34 |
| Yes | 35 | 12.6 | 14 | 15.6 | 21 | 11.2 | |
| No | 242 | 87.4 | 76 | 84.4 | 166 | 88.8 | |
| Prior cancer treatment | | | | | | | .31 |
| Yes | 31 | 11.2 | 13 | 14.4 | 18 | 9.6 | |
| No | 246 | 88.8 | 77 | 85.6 | 169 | 90.4 | |
| KPS, % | | | | | | | .41 |
| 90 to 100 | 187 | 68.0 | 64 | 71.9 | 123 | 66.1 | |
| < 90 | 88 | 32.0 | 25 | 28.1 | 63 | 33.9 | |
| 80 to 100 | 264 | 96.0 | 87 | 97.8 | 177 | 95.2 | .51 |
| < 80 | 11 | 4.0 | 2 | 2.2 | 9 | 4.8 | |
| T stage | | | | | | | .42 |
| 1 | 1 | 0.36 | 1 | 1.1 | 0 | 0 | |
| 2 | 17 | 6.1 | 5 | 5.6 | 12 | 6.4 | |
| 3 | 68 | 24.6 | 25 | 27.8 | 43 | 23.0 | |
| 4 | 191 | 68.9 | 59 | 65.6 | 132 | 70.6 | |
| N stage | | | | | | | .097 |
| N0 | 116 | 41.9 | 37 | 41.1 | 79 | 42.3 | |
| N1 | 106 | 38.3 | 41 | 45.6 | 65 | 34.8 | |
| Nx | 55 | 19.9 | 12 | 13.3 | 43 | 23.0 | |
| M stage | | | | | | | .75 |
| M0 | 266 | 96.0 | 86 | 95.6 | 180 | 96.3 | |
| Mx | 11 | 4.0 | 4 | 4.4 | 7 | 3.7 | |
| CA19-9 | | | | | | | |
| ≥ 1,000 | 72 | 26.3 | 19 | 21.4 | 53 | 28.6 | .24 |
| < 1,000 | 202 | 73.7 | 70 | 78.6 | 132 | 71.4 | |

Abbreviations: CA19-9, cancer antigen 19-9; FU, fluorouracil; KPS, Karnofsky performance status; RT, radiotherapy; SD, standard deviation; SOC, standard of care.

## RESULTS

### Patients

From April 5, 2005, to March 30, 2010, 473 patients were screened. Of these, 304 were randomly assigned as depicted in Figure 1 to yield 187 patients in the SOC + TNFerade arm and 90 in the SOC arm who were included in the modified intention-to-treat analysis. Median follow-up was 9.1 months (range, 0.1 to 50.5 months) for all patients and 8.1 months for patients still living at study termination. Of 39 participating institutions, 11 (28%) enrolled ≥ 10 patients. There were no significant differences in demographic or baseline disease characteristics between groups (Table 1).

### Efficacy

*Overall survival.* Median survival stratified by site and KPS (≥ 80% and < 80%) was similar for SOC + TNFerade and SOC (10.0 *v* 10.0 months; HR, 0.90; 95% CI, 0.66 to 1.22; $P = .26$; Table 2; Fig 2A). In the SOC + TNFerade arm, 16 patients (8.6%) died within 3 months following CRT as opposed to 12 patients (13.3%) in the SOC arm.

Multivariate analysis identified five baseline characteristics prognostic for survival: age, M stage (M0 *v* Mx), prior cancer history, prior cancer treatment, and baseline plasma CA19-9 (Appendix Table A1, online only). When adjusted for demographic and clinical factors, treatment with TNFerade remained a nondeterminant of overall survival (HR, 0.85; 95% CI, 0.61 to 1.19; $P = .34$). Among patients treated with SOC + TNFerade, no difference in median survival was observed for TNFerade delivery by PTA compared with EUS (9.4 *v* 11.5 months, respectively; HR, 1.06; 95% CI, 0.77 to 1.47; $P = .71$;Table 3; Fig 3A). Eighteen patients (10%) in the SOC + TNFerade arm went on to have successful surgical resection versus 10 patients (11%) in the SOC arm ($P = .68$) with margin-negative resections occurring in 78% and 60% of patients, respectively ($P = .40$).

*PFS.* Median PFS was similar between SOC + TNFerade and SOC arms (6.8 *v* 7.0 months, respectively; HR, 0.96; 95% CI, 0.69 to 1.32; $P = .51$; Fig 2B). Among patients treated on the SOC + TNFerade arm, multivariate analysis showed TNFerade injection by EUS rather than PTA to be a risk factor for inferior PFS (HR, 2.08; 95% CI, 1.06 to 4.06; $P = .032$; Fig 3B) after adjusting for age, sex, KPS, CA19-9, T stage, N stage, M stage, prior cancer history, prior cancer treatment, and study sites.

*Time to radiologic progression.* Median time to any radiologic progression was no different between SOC + TNFerade and SOC arms (11.6 *v* 10.8 months; HR, 1.07; 95% CI, 0.71 to 1.62; $P = .82$; Fig 2C). Within 3 months of CRT initiation, 47 patients (25.1%) in the SOC + TNFerade arm and 27 patients (30.0%) in the SOC arm developed metastatic disease.

Among patients treated with SOC + TNFerade, median time to progression was shorter for TNFerade administration by EUS compared with PTA (10.0 *v* 14.1 months; HR, 1.52; 95% CI, 1.00 to 2.33; $P = .05$; Table 3; Fig 3C). Multivariate analysis confirmed injection by EUS versus PTA as a risk factor for decreased time to progression (HR, 2.46; 95% CI, 1.15 to 5.28; $P = .02$) after adjusting for patient characteristics and clinical factors. Univariate analyses showed trends toward decreased time to distant progression (HR, 1.52; 95% CI, 0.93 to 2.47; $P = .09$) and local progression (HR, 1.99; 95% CI, 0.92 to 4.32; $P = .08$) for TNFerade administration via EUS (Table 3; Figs 3D and 3E).

*Radiologic response rates.* Tumor response was assessed by independent radiologic review for 147 patients (97 [51.9%] of 187 patients in the SOC + TNFerade arm and 50 [55.6%] of 90 patients in the SOC arm). There was no difference in response rates within this subset of patients (Table 2). Independent radiologic review was not completed for the remaining randomly assigned patients because of discontinuation of the trial for futility after planned interim analysis.

**Table 2.** Summary of Efficacy Measures by Intention-to-Treat Group

| Outcome | SOC + TNFerade (n = 187) No. | SOC + TNFerade (n = 187) % | SOC + TNFerade (n = 187) 95% CI | SOC (n = 90) No. | SOC (n = 90) % | SOC (n = 90) 95% CI | HR* | 95% CI | P* |
|---|---|---|---|---|---|---|---|---|---|
| Overall survival, months† | | | | | | | 0.90 | 0.66 to 1.22 | .26 |
| Median | 10.0 | | 8.8 to 11.6 | 10.0 | | 7.6 to 11.2 | | | |
| 12-month survival rate | | 41.0 | 33.5 to 48.3 | | 36.7 | 26.3 to 47.1 | | | |
| 18-month survival rate | | 23.1 | 16.9 to 29.9 | | 17.7 | 10.1 to 27.0 | | | |
| 24-month survival rate | | 11.3 | 0.07 to 17.1 | | 10.3 | 0.05 to 18.6 | | | |
| Progression-free survival, months | | | | | | | 0.96 | 0.69 to 1.32 | .51 |
| Median | 6.8 | | 5.5 to 8.8 | 7.0 | | 4.6 to 9.2 | | | |
| Time to radiologic progression, months | | | | | | | 1.07 | 0.71 to 1.62 | .82 |
| Median | 11.6 | | 9.6 to 14.1 | 10.8 | | 7.3 to 17.2 | | | |
| Level of radiologic response‡ | 97 | | | 50 | | | | | .74 |
| Complete | 0 | | | 0 | | | | | |
| Partial | 8 | 8.2 | | 6 | 12.0 | | | | |
| Stable disease | 72 | 74.2 | | 37 | 74.0 | | | | |
| Progressive disease | 17 | 17.5 | | 7 | 14.0 | | | | |

Abbreviations: HR, hazard ratio; SOC, standard of care.

*According to the protocol, the comparison of the treatment groups should be stratified by site and Karnofsky performance status (KPS; ≥ 80% and < 80%). A log-rank test was used to compare the two treatments stratified by site and KPS, and the HR was estimated from a Cox regression model adjusting for site and KPS status.

†Median and 12-month, 18-month, and 24-month survival rates were estimated by using the Kaplan-Meier method.

‡Radiologic response was evaluated for a subgroup of 147 patients (97 from the SOC + TNFerade arm and 50 from the SOC arm) by an independent central reading laboratory that was blinded to treatment assignment.

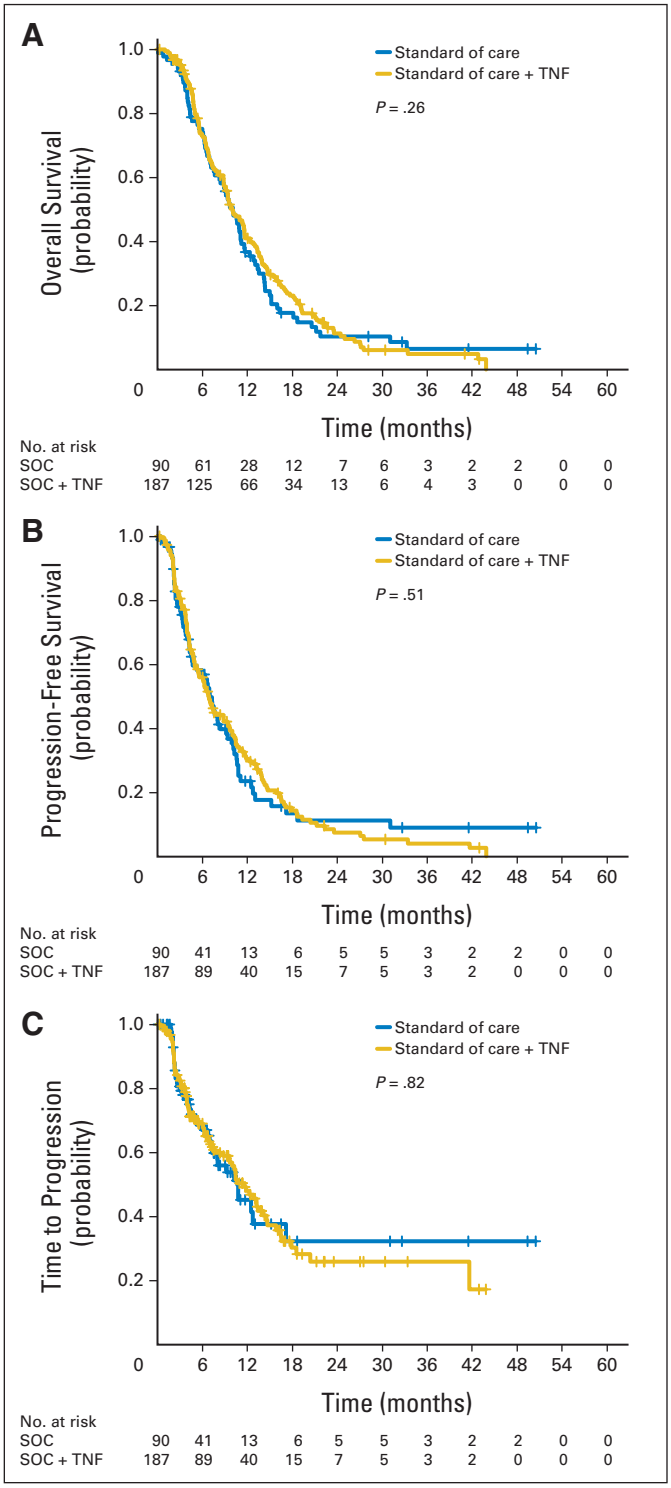


**Fig 2.** Kaplan-Meier curves depicting (A) overall survival, (B) progression-free survival, and (C) time to radiologic progression by modified intention-to-treat groups. *P* values given in each panel represent the significance level obtained when groups were compared by univariate analysis. SOC, standard of care; TNF, TNFerade.

### *Treatment Compliance*

In the SOC + TNFerade arm, 82.4% completed combined TNFerade and CRT, whereas 81.1% in the SOC arm completed CRT ($P = .87$). Of patients treated with SOC + TNFerade, 51.3% received TNFerade by EUS and 48.7% received TNFerade by PTA. EUS resulted in successful dose delivery in 450 (96.8%) of 465 attempts, and PTA resulted in successful dose delivery in 428 (98.2%) of 436 attempts ($P = .21$). Of patients who received TNFerade by EUS, 91.7% completed treatment with the prescribed five doses of TNFerade versus 91.2% of those who received TNFerade by PTA ($P = .99$).

At least 1 day of radiation treatment was missed by 121 patients (64.7%) in the SOC + TNFerade arm versus 56 patients (62.2%) in the SOC arm ($P = .69$; Appendix Table A2, online only). Only a minority of patients missed days of radiation treatment because of adverse events (27 [22.3%] of 121 in the SOC + TNFerade arm and seven [12.5%] of 56 in the SOC arm; $P = .15$), with the majority of missed days resulting from radiation facility closures because of holidays, equipment failure, or maintenance. All patients considered to have completed CRT made up all missed treatments on non-weekdays or at the end of therapy.

Gemcitabine administration was similar, with 68.4% versus 70.0% receiving maintenance therapy ($P = .89$) consisting of 11.0 ± 9.2 doses in the SOC + TNFerade arm and 12.5 ± 10.7 doses in the SOC arm on average ($P = .34$). In the SOC + TNFerade arm, 21.9% received erlotinib versus 15.6% in the SOC arm ($P = .26$), and mean duration of therapy was 2.6 ± 2.8 versus 1.8 ± 2.3 months, respectively ($P = .43$). Average erlotinib dose was 105 ± 43 mg for the SOC + TNFerade arm and 104 ± 31 mg for the SOC arm ($P = .91$).

### *Safety and Toxicity*

The overall incidence of definite or probable treatment-related grade 2 to 4 AEs was 75.9% for the SOC + TNFerade arm and 65.6% for the SOC arm ($P = .08$). A breakdown of AE by category (gastrointestinal, hematologic, nongastrointestinal/nonhematologic) and highest grade experienced per patient is provided in Table 4, along with the most commonly occurring toxicities within each category. AEs reported for patients receiving TNFerade were predominantly grade 1 to 2 in severity and either gastrointestinal or constitutional. Grade 1 to 2 pyrexia, chills, rigors, and sweats occurred at higher frequency for the patients in the SOC + TNFerade arm than for those in the SOC arm (81.7% *v* 14.3%; $P < .001$), but the rate of these constitutional toxicities at the grade 3 to 4 level was not significantly different between arms (3.2% *v* 1.1%; $P = .43$). All grade 3 to 4 AEs are summarized in Appendix Table A3 (online only) and occurred at similar frequencies in the SOC + TNFerade arm versus the SOC arm (all $P > .05$).

More patients receiving SOC + TNFerade experienced grade 2 to 4 AEs related to CRT (50.8% *v* 37.8% for SOC; $P = .05$; Appendix Table A2). Grade 3 to 4 laboratory abnormalities occurred at similar rates (72.7% for the SOC + TNFerade arm *v* 67.8% for the SOC arm; $P = .40$), with the majority in both groups occurring during gemcitabine maintenance therapy. Rates of grade 2 to 4 treatment-related toxicity were similar for EUS and PTA administration of TNFerade ($P = .80$).

There was a trend toward greater overall incidence of serious adverse events (SAEs) from any cause in the SOC + TNFerade arm (80.2%) compared with the SOC arm (70.0%; $P = .07$). The majority of SAEs in both arms were due to disease progression with only 25.7% of SOC + TNFerade patients and 16.7% of SOC patients experiencing treatment-related SAEs ($P = .13$). SAEs qualifying as thrombotic events occurred at similar rates between arms (9.6% for SOC + TNFerade *v* 11.9% for SOC; $P = .68$).

  

**Table 3.** Summary of Efficacy Measures Among Patients in the SOC + TNFerade Arm by TNFerade Delivery Method

| | EUS (n = 95) | | PTA (n = 91) | | | | |
|---|---|---|---|---|---|---|---|
| Outcome (months)* | Median | 95% CI | Median | 95% CI | HR† | 95% CI | P† |
| Overall survival | 11.5 | 9.1 to 13.3 | 9.4 | 7.3 to 11.6 | 1.06 | 0.77 to 1.47 | .71 |
| Progression-free survival | 6.8 | 5.0 to 9.6 | 6.8 | 4.8 to 10.4 | 1.37 | 0.97 to 1.92 | .07 |
| Time to radiologic progression | 10.0 | 7.2 to 12.3 | 14.1 | 7.0 to 41.6 | 1.52 | 1.00 to 2.33 | .05 |
| Time to distant metastasis | 11.9 | 8.8 to 20.4 | 17.9 | 14.1 to N/A | 1.52 | 0.93 to 2.47 | .09 |
| Time to local progression | 16.5 | 11.8 to N/A | N/A | 18.5 to N/A | 1.99 | 0.92 to 4.32 | .08 |

Abbreviations: EUS, endoscopic ultrasound guided (transgastric/transduodenal approach); HR, hazard ratio; N/A, not achieved; PTA, percutaneous transabdominal approach; SOC, standard of care.
*Median survival and times to progression were estimated by using the Kaplan-Meier method.
†HR and corresponding *P* value were computed on the basis of a univariate Cox regression model.

## DISCUSSION

To the best of our knowledge, this is the largest prospective study conducted to date in patients with LAPC and the first randomized trial to examine the efficacy of gene transfer therapy in pancreatic cancer. It is unclear why adding TNFerade to SOC failed to improve survival. Given the potency exhibited by TNFerade in preclinical studies,[17,29,32-35] we find it difficult to attribute the lack of survival benefit solely to unsuccessful tumor cell killing, although we cannot rule out this possibility. The following are alternative explanations: (1) delivery of TNFerade was ineffective, (2) the radiation-inducible promoter was not successfully activated, or (3) the majority of patients succumbed to metastatic disease and therefore more aggressive local therapy had minimal influence on the natural history of the disease. Herein, we attempt to evaluate each possibility in succession.

Pancreatic tumors have been previously described as exceedingly fibrous, hypoxic masses,[42-44] the composition of which may impede delivery of sufficient TNFerade throughout the tumor entirety to have a significant effect on tumor response. During TNFerade injections, difficulty penetrating the tumor capsule was noted. It is, therefore, quite possible that large regions of tumor were not exposed to TNFerade. Problematic penetration of the tumor capsule has also been described during pancreatic biopsies and fiducial implantation.[45-48] This rationale may also explain why PTA injection produced favorable disease progression outcomes compared with EUS. Considering the difficulty of achieving homogeneous dispersion of TNFerade throughout large, fibrotic pancreatic tumors, it is possible that greater variability existing in EUS operator skill across the 39 participating institutions compared with the more straightforward PTA technique may have resulted in reduced efficacy.

It does appear that the radiation-inducible promoter was activated during radiotherapy, as demonstrated by increased pyrexia and flu-like systemic symptoms in the SOC + TNFerade arm. Although more common for patients in the SOC + TNFerade arm, the vast majority of these constitutional symptoms were grade 1 to 2 in severity and were substantially reduced compared with those described in previous studies of tumor necrosis factor alpha.[22-28]

Many patients in our study (33.7% in the SOC + TNFerade arm and 43.3% in the SOC arm) either developed metastases or died during the first 3 months following initiation of CRT. These findings are consistent with other studies in which 20% to 30% of patients with LAPC developed metastatic disease soon after chemotherapy or CRT.[49,50] Therefore, several centers now treat LAPC with 2 to 4 months of chemotherapy and proceed with CRT if there is no evidence of systemic progression. This approach may protect against metastatic disease and "select" patients likely to benefit from aggressive local therapy.

We did not observe any difference in local response with the addition of TNFerade. One challenge of treating LAPC is determining whether CRT was effective. For example, following CRT, most locally advanced tumors are stable on CT imaging and, consequently, are not surgically explored. In the case of borderline resectable disease, however, tumors are often explored as long as there is no local or distant progression following CRT. Interestingly, several of these patients, up to 15% in some series, are found to have complete pathologic responses despite manifesting no change on CT imaging.[51,52] Furthermore, a majority undergo margin- and node-negative resections, suggesting that some tumors respond to therapy without clear radiographic evidence.[53,54] In this study, only 10% of patients underwent resection, so definitive conclusions cannot be drawn. MRI and/or positron emission tomography/CT imaging following CRT may better predict tumor response and enhance determination of optimal surgical candidates.[55,56]

It is encouraging that compliance was similar between arms despite the aggressive treatment protocol. This finding demonstrates that weekly EUS or PTA injection of TNFerade or future drugs in combination with CRT is feasible. In aggregate, grade 2 to 4 toxicity was greater for patients in the SOC + TNFerade arm. However, the increased toxicity did not preclude completion of treatment, suggesting that conditional expression of TNF-$\alpha$ under the Egr-1 promoter adequately limited systemic toxicity. This represents a substantial improvement from previous studies of TNF-$\alpha$ (eg, under a constitutive promoter) in which grade 3 to 4 toxicity related to TNF-$\alpha$ was dose-limiting or exceedingly high.[20-29]

### *Implications for Future Treatment*

For patients with LAPC, weekly EUS or PTA injection in combination with CRT is feasible. Integrating a conditional promoter into the therapeutic gene transfer vector is a viable method

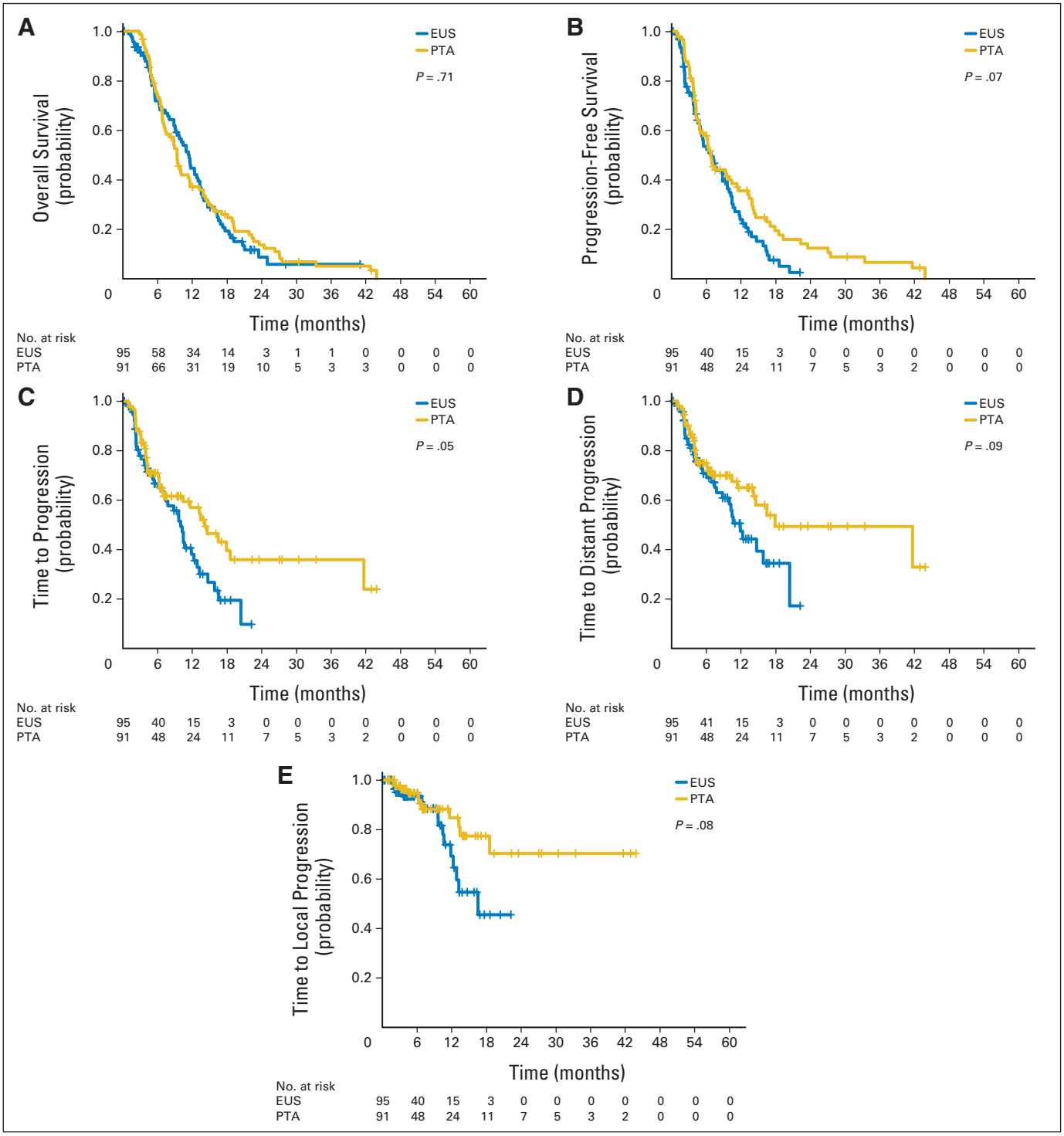


**Fig 3.** Kaplan-Meier curves depicting (A) overall survival, (B) progression-free survival, (C) time to any radiologic progression, (D) time to distant radiologic progression, and (E) time to local radiologic progression by TNFerade delivery method among patients treated on the standard of care + TNFerade arm. *P* values given in each panel represent the significance level obtained when groups were compared by univariate analysis. EUS, endoscopic ultrasound guided (transgastric/transduodenal approach); PTA, percutaneous transabdominal approach.

of limiting severe systemic toxicity. Future studies requiring intratumoral drug injection may benefit from consideration of techniques to achieve better pancreatic tumor penetration and drug distribution given the fibrous, hypoxic nature of these tumors. In addition, future studies testing aggressive local therapies should consider enrolling patients with borderline resectable tumors, which are generally smaller and more likely to be explored despite minimal radiographic change. Since tumor response by CT alone is limited following CRT, the utility of other methods, such as positron emission tomography/CT and MRI, should be investigated.

  

**Table 4.** Categorization of Adverse Events by Toxicity Type and Highest Grade Experienced Per Patient

| Toxicity Type | SOC + TNFerade No. | SOC + TNFerade % | SOC No. | SOC % | *P** |
|---|---|---|---|---|---|
| GI† | | | | | |
| Grade 2 | 52 | 28.0 | 16 | 17.6 | .07 |
| Grade 3 | 31 | 16.7 | 10 | 11.0 | .28 |
| Grade 4 | 3 | 1.6 | 0 | 0.0 | .55 |
| Hematologic‡§ | | | | | |
| Grade 2 | 20 | 10.8 | 12 | 13.2 | .55 |
| Grade 3 | 50 | 26.9 | 23 | 25.3 | .89 |
| Grade 4 | 10 | 5.4 | 9 | 9.9 | .21 |
| Non-GI/nonhematologic¶ | | | | | |
| Grade 2 | 41 | 22.0 | 16 | 17.6 | .43 |
| Grade 3 | 20 | 10.8 | 5 | 5.5 | .18 |
| Grade 4 | 2 | 1.2 | 2 | 2.2 | .60 |

Abbreviation: SOC, standard of care.
**P* values are computed by using Fisher's exact test.
†In descending order of frequency, the most commonly occurring GI toxicities were nausea/vomiting, abdominal pain, and anorexia in the SOC + TNFerade arm versus nausea/vomiting, diarrhea, and anorexia in the SOC arm.
‡In both arms, the majority of hematologic toxicities (> 85%) took place during gemcitabine-based maintenance therapy following chemoradiotherapy.
§In descending order of frequency, the most commonly occurring hematologic toxicities were neutropenia, thrombocytopenia, anemia, and lymphopenia in both the SOC + TNFerade and SOC arms.
¶In descending order of frequency, the most commonly occurring non-GI/nonhematologic toxicities were fatigue, chills/rigors/sweats, pyrexia, and dehydration in the SOC + TNFerade arm versus fatigue, dehydration, dermatitis, and hypokalemia in the SOC arm.

In conclusion, TNFerade administered in the fashion tested here was shown to be safe, but not effective in prolonging survival in patients with unresectable LAPC. Given the high incidence of subsequent metastatic disease in patients with LAPC, patients should receive up-front aggressive chemotherapy to select patients who are more likely to benefit from local therapy.

## AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

*Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.*
**Employment or Leadership Position:** None **Consultant or Advisory Role:** Kenneth J. Chang, GenVec (C) **Stock Ownership:** None **Honoraria:** None **Research Funding:** Kenneth J. Chang, GenVec **Expert Testimony:** None **Other Remuneration:** None

## AUTHOR CONTRIBUTIONS

**Conception and design:** Joseph M. Herman, Marcia I. Canto, Daniel A. Laheru
**Administrative support:** Hongyan Cai
**Collection and assembly of data:** Joseph M. Herman, Kenneth J. Chang, Gretchen E. Taylor, Ross C. Donehower, Hongyan Cai, Dionne T. Savage, Marcia I. Canto, Jason Klapman, Tony Reid, Raj J. Shah, Sarah E. Hoffe, Alexander Rosemurgy, Daniel A. Laheru
**Data analysis and interpretation:** Joseph M. Herman, Aaron T. Wild, Hao Wang, Phuoc T. Tran, Timothy M. Pawlik, Mark A. Ziegler, Christopher L. Wolfgang, Daniel A. Laheru
**Manuscript writing:** All authors
**Final approval of manuscript:** All authors

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