表1　GEM併用およびS-1併用放射線療法第II相試験

報告者	n	化学療法	線量	奏効率	MST	主な急性毒性*
Okusaka[5]	42	GEM 250 mg/m²/w	50.4 Gy	21%	9.5か月	白血球(52%) 食欲低下(57%)
Shibuya[6]	21	GEM 250 mg/m²/w	54 Gy	29%	16.6か月	白血球(52%) 貧血(14%)
Small[7]	39	GEM 1,000 mg/m² Day 1, 8, 15	36 Gy	5.1%	NA	好中球(13%) 悪心(10%)
Kim[11]	25	S-1 80 mg/m² Day 1～14, 22～35	50.4 Gy	24%	12.9か月	食欲低下(20%)
Sudo[8]	34	S-1 80 mg/m² Day 1～14, 22～35	50.4 Gy	41%	16.8か月	食欲低下(24%) 悪心(12%)
Shinchi[10]	50	S-1 80 mg/m² Day 1～21	50 Gy	30%	14.3か月	－
Ikeda[9]	60	S-1 80 mg/m² 照射当日	50.4 Gy	27%	16.2か月	白血球(10%)

MST：生存期間中央値，w：week．＊Gr3以上

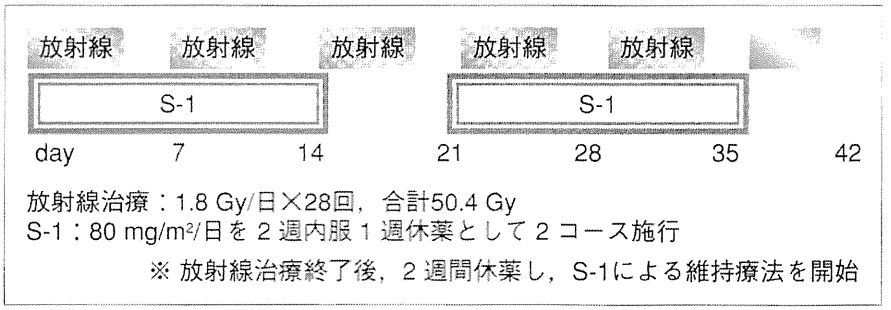

図1　S-1併用放射線療法

開発が積極的に行われている．

1．GEM併用放射線療法

GEMはBurrisらの報告以後，遠隔転移を有する進行膵癌に対する標準的治療としての地位を確立してきたが，同時に強力な放射線増感作用を有することが知られている．当初，GEMを併用した化学放射線療法に期待が集まり，多くの第Ⅰ相試験が行われた．しかし，放射線と併用する場合，GEMの投与量は週2回投与で40～50 mg/m²/day，週1回投与で250～440 mg/m²/dayと全身化学療法の投与量(1,000 mg/m²/day)よりも減量する必要があり，GEM自体の抗腫瘍効果はあまり期待できない[2)～6)]．第II相試験の結果は生存期間中央値(MST)9.5～16.6か月と報告されるが[5)6)]，血液毒性や消化器毒性など有害事象の頻度は概して5-FU系薬剤を併用した放射線療法と比べ高い傾向にある(表1)．このほか，放射線照射野を縮小させ全身化学療法のGEM投与量(1,000 mg/m²/day)を併用する試みも報告されており，第Ⅰ相試験では36 Gy(2.4 Gy×15回)が推奨線量とされる．第II相試験では切除可能膵癌も含まれるデータであるが，1年生存率73%と報告される[7)]．

2．S-1併用放射線療法

S-1は5-FU系の経口剤であり，遠隔転移を有する進行膵癌および局所進行膵癌においてGEM単剤に対する非劣性が証明されたはじめての薬剤である．このS-1を利用した放射線治療は本邦を中心に開発が行われ，有効な治療法として期待される．S-1は5-FUの放射線増感作用に加え，構成成分の一つであるギメラシルにも増感作用があることが報告されている．また，S-1はGEMに対する非劣性が証明された薬剤であり，全身化学療法としての働きも期待できる．

S-1併用放射線療法はこれまで3つの第Ⅰ相試験が報告されるが，標準的なスケジュールは確立されていない．図1に筆者らの報告したS-1併用放射線療法の概要を示すが，第Ⅰ相試験に基づき，50.4 Gyの放射線治療に対しS-1の常用1日

表2　導入化学療法を併用した化学放射線療法の臨床試験

報告者	n	導入療法	化学放射線療法	MST
Mishra(2005)[14]	20	GEM＋CPT-11	GEM＋50.4 Gy	8.8か月
Moureau-Zabotto(2008)[15]	59	GEM＋Ox	5-FU＋Ox＋55 Gy	12.2か月
Nakachi(2010)[16]	20	GEM＋S-1	GEM＋30 Gy	14.4か月
Ch'ang(2011)[17]	50	GEM＋Ox＋5-FU＋LV	GEM＋50.4 Gy	14.5か月
Crane(2011)[18]	69	GEM＋Ox＋cetuximab	Capecitabine＋cetuximab＋50.4 Gy	19.2か月
Kim(2012)[19]	37	GEM＋CDDP	Capecitabine＋55.8 Gy	16.8か月

MST：生存期間中央値，Ox：オキサリプラチン，LV：ロイコボリン

量である80 mg/m²の併用が可能である．また，放射線治療終了後にはS-1単剤による維持化学療法を行うことで，膵原発巣のみならず潜在的な遠隔転移のコントロールも意図している．第II相試験では奏効割合41%，MST 16.8か月と良好な成績であった[8]．また，このほかにも3つの第II相試験が報告されており，MST 12.9～16.2か月と同様の成績が報告されている[9)~11)]．

欧米ではS-1の代わりに他の5-FU系経口剤であるカペシタビンを使用した化学放射線療法が行われている．最近報告されたカペシタビン併用放射線療法とGEM併用放射線療法の無作為化第II相試験ではカペシタビン併用群で良好な全生存期間(15.2か月 vs. 13.4か月，$P=0.012$)と低い血液毒性(grade 3～4：0% vs. 18%，$P=0.008$)が示されており，GEM併用放射線療法に対する優位性が示唆される[12]．

3．導入化学療法を用いた化学放射線療法

画像診断のみで局所進行膵癌と診断された症例には一定の割合で微小な腹膜播種や肝転移などの高度進行癌が含まれていることが知られている．これらの患者では局所治療を主体とした放射線治療の意義は少なく，放射線治療の対象から除外する必要がある．また，切除可能膵癌であっても，根治切除後にしばしば遠隔転移再発をきたすことを考えると，局所進行膵癌の治療は潜在的な遠隔転移のコントロールに配慮した治療ストラテジーの確立が重要である．こうした観点から，まず全身化学療法を導入し，一定期間遠隔転移再発のない患者集団を選別し，化学放射線療法を行う治療戦略の有用性が報告される．

HuguetらはGERCOR第II・III相試験に登録された181例の局所進行膵癌症例のうち，導入化学療法により3か月後のCTで遠隔転移をきたさなかった128例について後方視的に検討した[13]．その結果，化学放射線療法を導入した72例では化学療法を継続した56例と比較し，生存期間は有意に良好であった(15か月 vs. 11.7か月，$P=0.0009$)．このほか，これまで多数の第II相試験が行われ，生存期間中央値は8.8～19.2か月と報告される(表2)[14)~19)]．無作為化比較試験による十分なエビデンスはないが，National Comprehensive Cancer Network(NCCN)の膵癌ガイドライン2014年版においても，十分な化学療法を行い遠隔転移の出現しない症例に対し化学放射線療法を行うことが好ましいと記載されている．

反対に，2013年のASCO(American Society of Clinical Oncology)ではLAP07試験の最終解析結果が報告され，GEMまたはGEM＋エルロチニブによる導入化学療法後のカペシタビン併用化学放射線療法に対し否定的な結果が示された[20]．本試験ではGEMまたはGEM＋エルロチニブ療法を施行した442例のうち，病勢コントロールの得られた269例をランダム化し，カペシタビン併用放射線療法または化学療法を継続する2群に割り付け登録を行った．両群の生存期間中央値は15.3か月 vs. 16.5か月($P=0.83$)と有意差を認めなかった．

局所進行膵癌に対する全身化学療法

GEMの登場以後，遠隔転移例および局所進行例を対象に多くの第III相試験が行われたが，この中で局所進行膵癌に対するGEMベースの化学療法の治療成績はおよそ10か月程度である(表3)[21)~23)]．これは従来行われてきた5-FU併用放射線療法と同等の治療成績であり，GEMによる化学療法を局所進行膵癌治療の第1選択とする施設も少な

表3 局所進行膵癌に対する全身化学療法

報告者	化学療法	n	デザイン	MST
Rocha Lima(2004)[21]	GEM	24	Phase 3	11.7か月
	GEM＋CPT-11	27	subgroup analyses	9.8か月
Louvet(2005)[22]	GEM	47	Phase 3	10.3か月
	GEM＋Ox	51	subgroup analyses	10.3か月
Heinemann(2006)[23]	GEM	20	Phase 3	10.4か月
	GEM＋CDDP	19	subgroup analyses	10.3か月
Ishii(2010)[24]	GEM	50	Phase 2	15か月
Fukutomi(2012)[25]	GEM	66	Phase 3(GEST study)	12.7か月
	GEM＋S-1	68	subgroup analyses	15.9か月
	S-1	68		13.8か月
Metges(2014)[26]	FOLFIRINOX	59	retrospective	11.2か月

MST：生存期間中央値，Ox：オキサリプラチン

くない．本邦では，Ishiiらが局所進行膵癌に対するGEM単剤の第II相試験を行い，MST 15か月と良好な成績を報告している[24]．また，GEST試験における局所進行膵癌に対する治療成績はGEM群12.7か月，S-1群13.8か月，GEM＋S-1療法群15.9か月といずれにおいても10か月を越える成績が示されている[25]．

近年，遠隔転移例においてFOLFIRINOX療法やGEM＋ナブパクリタキセル療法などのGEM単剤よりも有効性の高い治療法が報告されるが，これらの治療法の局所進行膵癌に対する有効性も大いに期待される．FOLFIRINOXについては局所進行膵癌59例のレトロスペクティブな解析が行われており，生存期間中央値11.2か月と報告されるが[26]，前向きのデータは乏しく，今後の検討課題である．

全身化学療法か？ 化学放射線療法か？

最新の膵癌診療ガイドライン(2013年版)によれば切除不能局所進行膵癌に対する治療は化学療法単独または化学放射線療法の両者が推奨されるが(グレードA)，化学療法と化学放射線療法の優劣については十分なエビデンスがないのが現状である．近年，GEM単剤と化学放射線療法との第III相試験が2つ報告され，相反する結果が示されたが，いずれも予定登録数に達せず中断されており，正当な評価は難しい．

一方，局所制御という点では化学放射線療法の方がより強力である可能性がある．レトロスペクティブな検討であるが，Carbonellらは過去に報告された術前化学放射線療法の成績を集計し，494例中218例に膵切除が行われ，28例(5.7%)に病理学的CRが得られたと報告している[27]．一方，化学療法単独では病理学的CRの報告は少なく，放射線治療の追加により稀ではあるが，病理学的CRが得られる可能性が示唆される．実際，局所進行膵癌に対する化学放射線治療により，長期生存例の存在が報告されている．われわれの検討でも化学放射線療法を行った局所進行膵癌82例(病理学的診断あり)において，10年の無増悪生存例を認めており，3年生存率17.5%，5年生存率6.7%と一部に非切除治療のみで長期生存が得られている[28]．

多くの局所進行膵癌において経過中に遠隔転移をきたすことを考えると，全身化学療法を重視した治療体系の構築は不可欠である．しかし，放射線治療の意義についてもこうした長期生存という視点からの検討も重要と考えられる．

奏効例に対する外科切除

近年，非切除治療の進歩により，局所進行膵癌においても奏効する症例が数多く経験される．このような場合，外科切除を勧めるか，または現在奏効している治療を継続するか悩ましいところである．

現在奏効している治療を継続した場合でも，いずれ増悪をきたす可能性が高く根治は難しい．一方，こうした奏効例の外科切除は一般に難易度が高く，手術侵襲による合併症のリスクを伴う．また，化学療法の中断により遠隔転移が出

現する可能性もある．術後早期に再発をきたすのではリスクをおかして外科切除を行う意味がない．

近年，切除不能(unresectable；UR)局所進行膵癌および切除可能膵癌の境界領域としてborderline resectable(BR)膵癌という概念が提唱され，積極的に術前治療および外科切除の成績が検討されている．MD Anderson Cancer CenterのKatzらは術前治療を行ったBR膵癌129例のうち85例(66%)に膵切除を行い，81例に根治切除可能であったと報告している．後方視的な検討ではあるが，切除不能例を含めた全129例の生存期間中央値は22か月と切除可能膵癌の治療成績と同等であった[29]．

また，より進行した状態であるUR例においても，外科切除の有用性を示唆する報告がある．Satoiらは切除不能膵癌(遠隔転移例を含む)を対象として化学療法または化学放射線療法により6か月以上SD, PR, CRを持続しえた159例を検討し，外科切除を追加した58例において，化学療法を継続したコントロール群101例よりも有意に生存期間が良好であったと報告している(生存期間中央値39.7か月 vs. 20.8か月，5年生存率34% vs. 10%，$P<0.0001$)[30]．高い5年生存割合は外科切除の有用性を示唆するが，本報告は後方視的な検討であり，コントロール群で腹膜播種が多く，PR・CR例が少ないなど両群の背景が異なっている．奏効したUR症例に対し外科切除を追加する意義については今後，無作為化比較試験による検証が必要である．

今後の展望

近年，局所進行膵癌の治療成績は向上しており，従来10か月程度であった生存期間中央値が，最近の報告では15か月を越える成績も多数報告される．2004年に本邦より報告されたステージIVa膵癌(以前の規約に基づいており，動脈浸潤例は含まれない)に対する外科切除群および5-FU併用放射線療法群の生存期間中央値がそれぞれ13か月および8.9か月であったことを考慮すると，その進歩の大きさがうかがえる．さらに最近では，FOLFIRINOX療法やGEM＋ナブパクリタキセル療法などの新しい治療法が開発されており，非切除治療の成績はさらなる発展が期待される．

今後，より高いレベルで遠隔転移および局所病変の制御が可能となれば，外科切除の意義もさらに高まるものと予想される．いかに根治切除率を高め，遠隔転移をきたし難い腫瘍を選別するかが課題であるが，確立された基準はない．UR症例について前述のSatoiらは8か月以上，奏効していた症例で切除成績が良好であったと報告しているが，現実的なアプローチとして興味深い[30]．また，近年腫瘍のSMAD4発現により，局所優位に進行する腫瘍を選別する試みも行われているが[31]，こうした局所進行膵癌の分子生物学的な病態解明はいまだ発展途上にある．最近の遺伝子解析技術は革新的な進歩を遂げており，今後さらなる発展に期待される．

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ORIGINAL ARTICLE

P53, hTERT, WT-1, and VEGFR2 are the most suitable targets for cancer vaccine therapy in HLA-A24 positive pancreatic adenocarcinoma

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Abstract Cancer vaccine therapy is one of the most attractive therapies as a new treatment procedure for pancreatic adenocarcinoma. Recent technical advances have enabled the identification of cytotoxic T lymphocyte (CTL) epitopes in various tumor-associated antigens (TAAs). However, little is known about which TAA and its epitope are the most immunogenic and useful for a cancer vaccine for pancreatic adenocarcinoma. We examined the expression of 17 kinds of TAA in 9 pancreatic cancer cell lines and 12 pancreatic cancer tissues. CTL responses to 23 epitopes derived from these TAAs were analyzed using enzyme-linked immunospot (ELISPOT), CTL, and tetramer assays in 41 patients, and factors affecting the immune responses were investigated. All TAAs were frequently expressed in pancreatic adenocarcinoma cells, except for adenocarcinoma antigens recognized by T cells 1, melanoma-associated antigen (MAGE)-A1, and MAGE-A3. Among the epitopes recognized by CTLs in more than two patients in the ELISPOT assay, 6 epitopes derived from 5 TAAs, namely, MAGE-A3, p53, human telomerase reverse transcriptase (hTERT), Wilms tumor (WT)-1, and vascular endothelial growth factor receptor (VEGFR)2, could induce specific CTLs that showed cytotoxicity against pancreatic cancer cell lines. The frequency of lymphocyte subsets correlated well with TAA-specific immune response. Overall survival was significantly longer in patients with TAA-specific CTL responses than in those without. P53, hTERT, WT-1, and VEGFR2 were shown to be attractive targets for immunotherapy in patients with pancreatic adenocarcinoma, and the induction of TAA-specific CTLs may improve the prognosis of these patients.

Keywords Epitope · Immunotherapy · Cytotoxic T lymphocyte (CTL) · Enzyme-linked immunospot (ELISPOT)

Abbreviations

CTL	Cytotoxic T lymphocyte
TAA	Tumor-associated antigen
ELISPOT	Enzyme-linked immunospot
MAGE	Melanoma-associated antigen
hTERT	Human telomerase reverse transcriptase
WT-1	Wilms tumor-1
VEGFR	Vascular endothelial growth factor receptor
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction

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Introduction

Pancreatic adenocarcinoma is the fourth leading cause of cancer death worldwide [1]. Despite recent advances in diagnostic techniques, pancreatic adenocarcinoma is diagnosed at an advanced stage in most patients and, consequently, the overall 5-year survival rate is <5 % [2]. Thus, the development of a new treatment option is needed to improve the prognosis of pancreatic cancer patients without toxicity.

Immunotherapy is one of the most attractive therapies as a new treatment procedure for melanoma and other solid tumors [3]. Recent technical advances have enabled the identification of various tumor-associated antigens (TAAs) [4–21]; however, few of their epitopes are inducers of cytotoxic T lymphocyte (CTL) responses against tumors [22]. Several kinds of epitope have also been identified in patients with pancreatic adenocarcinoma [23, 24]. However, previous studies focused on the identification and evaluation of a particular antigen, and different TAAs have not yet been compared simultaneously; therefore, little is known about which epitope is the most immunogenic and useful in eliciting clinical responses in pancreatic adenocarcinoma patients.

In the present study, we compared CTL responses with various TAA-derived epitopes in identical patients with pancreatic adenocarcinomas and examined the factors that affect immune responses. This approach provided information that is useful for selecting immunogenic TAAs and suitable patients and developing a new immunotherapy for pancreatic adenocarcinoma.

Materials and methods

Patients and clinical information

In this study, we examined 41 HLA-A24-positive patients with pancreatic adenocarcinoma and 14 healthy volunteers who were HLA-A24-positive, but did not have any cancers, as negative controls. Fine-needle biopsy, a surgical specimen, or autopsy was used for the pathological diagnosis of pancreatic adenocarcinoma in 18 patients. Diagnosis of the remaining 23 patients was achieved using the radiological findings of computed tomography and/or magnetic resonance imaging. We investigated patient background, treatment procedures, and outcomes.

Clinical information was obtained from the medical records of patients. We evaluated the tumor stage using TNM staging of the Union Internationale Contre Le Cancer (UICC) system (7th version) (UICC stage). The frequency of lymphocyte subsets was calculated by dividing the absolute lymphocyte count by the absolute leukocyte count. HLA typing of peripheral blood mononuclear cells (PBMCs) from patients and healthy volunteers was performed by the reverse sequence-specific oligonucleotide with polymerase chain reaction (PCR-RSSO). This study was approved by the Ethics Committees of Kanazawa University (No. 1237) and Kanazawa Medical Center (No. 17), and all patients gave written informed consent to participate in accordance with the Helsinki Declaration.

Table 1 Peptides used in this study

Peptide No.	TAA	Amino acid sequence	Reference
1	$ART1_{188}$	EYCLKFTKL	[14]
2	$ART4_{161}$	AFLRHAAL	[11]
3	$ART4_{899}$	DYPSLSATDI	[11]
4	Cyp-B_{109}	KFHRVIKDF	[7]
5	Cyp-B_{315}	DFMIQGGDF	[7]
6	Lck_{208}	HYTNASDGL	[8]
7	Lck_{488}	DYLRSVLEDF	[8]
8	MAGE-$A1_{135}$	NYKHCFPEI	[6]
9	MAGE-$A3_{195}$	IMPKAGLLI	[16]
10	$SART1_{690}$	EYRGFTQDF	[12]
11	$SART2_{899}$	SYTRLFLIL	[13]
12	$SART3_{109}$	VYDYNCHVDL	[21]
13	Her-2/neu_8	RWGLLLALL	[17]
14	$p53_{161}$	AIYKQSQHM	[18]
15	$p53_{204}$	EYLDDRNTF	[5]
16	$MRP3_{765}$	VYSDADIFL	[20]
17	$MRP3_{503}$	LYAWEPSFL	[20]
18	$hTERT_{461}$	VYGFVRACL	[4]
19	$hTERT_{324}$	VYAETKHFL	[4]
20	WT-1_{235}	CMTWNQMNL	[15]
21	$VEGFR2_{169}$	RFVPDGNRI	[19]
22	$VEGFR1_{1084}$	SYGVLLWEI	[10]
23	survivin$2B_{80}$	AYACNTSTL	[9]
24	HIV env_{584}	RYLRDQQLL	[25]
25	CMV $pp65_{328}$	QYDPVAALF	[26]

Synthetic peptides and preparation of PBMCs

The 23 epitopes derived from 17 different TAAs used in the present study are listed in Table 1. We selected epitopes that had previously been identified as HLA-A24-restricted and suggested to have immunogenicity in various cancers not restricted to pancreatic cancer [4–21]. Epitopes derived from the HIV envelope protein (HIV env_{584}) [25] and cytomegalovirus (CMV) pp65 (CMV$pp65_{328}$) [26] were also used to assess T cell responses. Peptides were synthesized at Mimotope (Melbourne, Australia), Sumitomo Pharmaceuticals (Osaka, Japan), COSMO BIO Co. (Tokyo, Japan), and Scrum Inc. (Tokyo, Japan). Purities were determined to be >80 % by analytical high-performance

liquid chromatography (HPLC). PBMCs were separated as described below; heparinized venous blood was diluted in phosphate-buffered saline (PBS) and loaded on Ficoll-Histopaque (Sigma, St. Louis, MO) in 50-ml tubes. After centrifugation at 2,000 rpm for 20 min at room temperature, PBMCs were harvested from the interphase, resuspended in PBS, centrifuged at 1,400 rpm for 10 min, and finally resuspended in complete culture medium consisting of RPMI (GibcoBRL, Grand Island, NY), 10 % heat-inactivated FCS (Gibco BRL), 100 U/ml penicillin, and 100 μg/ml streptomycin (Gibco BRL).

Cell lines

The HLA-A*2402 gene-transfected C1R cell line (C1R-A24) was cultured in RPMI 1640 medium containing 10 % FCS and 500 μg/ml hygromycin B (Sigma, St. Louis, MO), and K562 was cultured in RPMI 1640 medium containing 10 % FCS [27]. MiaPaca2, AsPC1, BxPC3, Panc-1, CAPAN1, and CAPAN2 were purchased from the American Type Culture Collection (VA, USA). YPK-1 and YPK-2 were kind gifts from Prof. Oka and Dr. Yoshimura (Yamaguchi University Graduate School of Medicine, Yamaguchi, Japan). PK-1 was provided by the RIKEN BRC through the National Bio-Resource Project of MEXT, Japan. Human pancreatic cancer cell lines were cultured in DMEM (GibcoBRL) or RPMI 1640 medium containing 10 % fetal calf serum (FCS). All media contained 100 U/mL penicillin and 100 μg/mL streptomycin.

RNA preparation and real-time PCR

The expression of TAA messenger RNA (mRNA) in human pancreatic cancer cell lines and pancreatic adenocarcinoma tissues was analyzed by real-time polymerase chain reaction (PCR). Cell lines were harvested, centrifuged, and washed with PBS, and total RNA was then isolated using Quick-Gene (Fuji Film, Tokyo). Total RNA from frozen pancreatic adenocarcinoma samples was isolated using a GenElute Mammalian Total RNA Miniprep Kit (Sigma-Aldrich) according to the manufacturer's protocol. cDNA was synthesized from 150 ng of total RNA using a high-capacity cDNA reverse transcription kit (PE Applied Biosystems, CA, USA) and was then mixed with TaqMan Universal Master Mix (PE Applied Biosystems) and each TaqMan probe. Primer pairs and probes for various TAAs and β-actin were obtained from the TaqMan assay reagents library. Thermal cycling conditions were 25 °C for 10 min, 37 °C for 120 min, and 85 °C for 1 min. cDNA was subjected to quantitative real-time PCR analyses targeting various TAAs and β-actin. Analyses were performed using the StepOne Real-Time PCR system and StepOne v2.0 software. Relative gene expression values were determined. Data are presented as fold differences in TAA expression normalized to the housekeeping gene β-actin as an endogenous reference.

Enzyme-linked immunospot assay (ELISPOT assay)

Ninety-six-well plates (Millititer, Millipore, Bedford, MA) were coated with anti-human interferon-γ (IFN-γ) (Mabtech, Nacka, Sweden) at 4 °C overnight and then washed 4 times with sterile PBS. The plates were then blocked with RPMI 1640 medium containing 5 % FCS for 2 h at room temperature. A total of 300,000 unfractionated PBMCs were added in duplicate cultures of RPMI 1640 containing 5 % FCS together with the peptides at 10 μg/ml. After 24 h, the plates were washed 8 times with PBS and incubated overnight with 100 μl of the biotin-conjugated anti-human IFN-γ antibody. After another 4 washes with PBS, streptavidin-AP was added for 2 h. Finally, the plates were washed again 4 times with PBS and developed with freshly prepared NBT/BCIP solution (Biorad, Hercules, CA). The reaction was stopped by washing with distilled water and drying at room temperature. Colored spots with fuzzy borders, which indicated the presence of IFN-γ-secreting cells, were counted. The number of specific spots was determined by subtracting the number of spots in the absence of the antigen. Responses were considered positive if 10 or more specific spots were detected and if the number of spots in the presence of an antigen was at least two-fold than that in its absence.

Peptide-specific CTL induction and cytotoxicity assay

Synthetic peptide-specific T cells were expanded from PBMCs in 96-well round-bottom plates (NUNC, Naperville, IL). Four hundred thousand cells/well were stimulated with synthetic peptides at 10 μg/ml, 10 ng/ml rIL-7, and 100 pg/ml rIL-12 (Sigma) in RPMI 1640 supplemented with 10 % heat-inactivated human AB serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. The cultures were restimulated with 10 μg/ml peptide, 20 U/ml rIL-2 (Sigma), and 10^5 mitomycin C-treated autologous PBMCs as feeder cells on days 7 and 14. One hundred microliters of RPMI medium with 10 % human Ab serum and rIL-2 at a final concentration of 10 U/ml were added to each well on days 4, 11, and 18. The cytotoxicity assay was conducted on day 22.

The C1R-A24 and human pancreatic cancer cell lines were used as target cells for the ^{51}Cr release assay. C1R-A24 cells were incubated overnight with 10 μg/ml synthetic peptides and labeled with 25 μCi of ^{51}Cr for 1 h. Pancreatic cancer cell lines were also labeled with 25 μCi of ^{51}Cr for 1 h without incubation with peptides. After three washes with PBS, target cells were plated at 3,000 cells/well in complete medium

in round-bottom 96-well plates. Unlabeled K562 (120,000 cells/well) was added to reduce non-specific lysis. Peptide-stimulated PBMCs were added at various effector-to-target ratios as indicated. Maximum release was determined by the lysis of ^{51}Cr-labeled targets with 5 % Triton X-100 (Sigma Chemical). Spontaneous release was <10 % of maximum release for all experiments, except for when it was <15 % when the target cells were human pancreatic cancer cell lines. Percent-specific cytotoxicity was determined using the following formula: 100 × (experimental release − spontaneous release)/(maximum release − spontaneous release), and specific cytotoxic activity was calculated as follows: (cytotoxic activity in the presence of the peptide) − (cytotoxic activity in the absence of the peptide). Specific cytotoxicity of more than 10 % was considered to be positive.

Tetramer staining and flow cytometry

TAA-specific tetramers were purchased from Medical Biological Laboratories Co., Ltd. (Nagoya, Japan). Tetramer staining was performed as described below. One million isolated PBMCs or peptide-specific CTLs pulsed with TAA-derived peptides were washed, resuspended in 200 μl of PBS without calcium or phosphate, and stained with 40 μg/ml tetrameric complexes and monoclonal antibodies against cell surface proteins for 30 min at room temperature. The following monoclonal antibodies were used: anti-CD8-APC (BD PharMingen, San Diego, CA), anti-CCR7-FITC, anti-CD45RA-PerCP, and tetramer-PE. Cells were washed, fixed with 0.5 % paraformaldehyde/PBS, and analyzed on a Becton–Dickinson FACSAria II system.

Statistical analysis

Fisher's exact test and unpaired Student's *t* test were used to analyze the effect of variables on immune responses in pancreatic cancer patients. Overall survival was calculated from the day of pancreatic cancer diagnosis until the date of death or the last day of the follow-up period. Cumulative survival proportions were calculated using the Kaplan–Meier method, and any differences were evaluated using the log-rank test. A p value of <0.05 was considered to be significant, and all the tests were two-sided. All statistical analyses were performed using the SPSS statistical software program package (SPSS version 11.0 for Windows).

Results

Patients

Patient characteristics are summarized in the Supplementary Table. The median age of patients was 72 years, and patients included 24 males (59 %). The main localization of the tumors was the pancreatic head in 39 % of patients and the pancreatic body or tail in 61 %. The majority of patients (93 %) had advanced-stage cancer, namely, UICC stage III or IV. Therapeutic procedures mainly involved chemotherapy consisting of protocols such as gemcitabine monotherapy, S-1 monotherapy, or a combination of both drugs. Only 11 patients received the best supportive therapy to relieve physical and spiritual pain. A total of 61 % of patients had died by the last day of the follow-up period, and the median overall survival time of patients was 7.2 months.

TAA expression in pancreatic cancer cell lines and human cancer tissues

We evaluated the expression of 17 different TAAs in 9 human pancreatic cancer cell lines using real-time PCR. Although differences were observed from cell to cell, TAAs were expressed in more than 40 % of pancreatic adenocarcinoma cell lines, except for adenocarcinoma antigens recognized by T cells (ART)1 (11 %) and ART4 (33 %) (Table 2). We then investigated TAA expression in 7 surgical and 5 autopsy specimens. The expression of most TAAs in pancreatic adenocarcinoma specimens was similar to or more frequent than that in human pancreatic cancer cell lines, except for melanoma-associated antigen (MAGE)-A1 and MAGE-A3 (Table 2).

Detection of TAA-specific T cells by IFN-γ ELISPOT analysis

IFN-γ ELISPOT responses were evaluated with PBMCs to determine how frequently T cells respond to TAA-derived peptides and control peptides in patients with pancreatic adenocarcinoma (Fig. 1a). Positive responses to at least one TAA-derived peptide were observed in 28 of 41 (68 %) patients. On the other hand, 14 of 23 (61 %) peptides were recognized by T cells obtained from at least one patient. $ART1_{188}$, $ART4_{161}$, $ART4_{899}$, lymphocyte-specific protein tyrosine kinase $(Lck)_{208}$, $MAGE\text{-}A3_{195}$, $p53_{161}$, human telomerase reverse transcriptase $(hTERT)_{461}$, $hTERT_{324}$, Wilms tumor $(WT)\text{-}1_{235}$, vascular endothelial growth factor receptor $(VEGFR)2_{169}$, and $VEGFR1_{1084}$ were recognized in more than two patients, which suggested that these peptides have the potential to be immunogenic. Peptides 24 $(HIVenv_{584})$ and 25 $(CMVpp65_{328})$ were recognized in 0 and 38 % of patients, respectively.

Peptides $ART4_{161}$, $ART4_{899}$, Cyclophilin B $(Cyp\text{-}B)_{315}$, Lck_{208}, $hTERT_{324}$, and $VEGFR1_{1084}$ were recognized in more than one healthy volunteer, and/or the percentage of positive responses was higher in healthy volunteers than in pancreatic adenocarcinoma patients, which indicated

Table 2 Expression of various TAAs mRNA in pancreatic cancer cell lines and pancreatic cancer tissues measured by real-time PCR

TAA	Primer	Positive cell lines/ cell lines tested *n* (%)	Positive specimens/ specimens tested *n* (%)
ART1	Hs00188841_m1	1/9 (11)	5/12 (42)
ART4	Hs00221465_m1	3/9 (33)	11/12 (92)
CypB	Hs00168719_m1	9/9 (100)	12/12 (100)
Lck	Hs00178427_m1	8/9 (89)	11/12 (92)
MAGEA1	Hs00607097_m1	4/9 (43)	1/12 (8)
MAGEA3	Hs00366532_m1	4/9 (43)	1/12 (8)
SART1	Hs00193002_m1	9/9 (100)	12/12 (100)
SART2	Hs00203441_m1	9/9 (100)	12/12 (100)
SART3	Hs00206829_m1	9/9 (100)	12/12 (100)
HER2/neu	Hs00170433_m1	9/9 (100)	12/12 (100)
p53	Hs00153340_m1	9/9 (100)	12/12 (100)
MRP3	Hs00358656_m1	9/9 (100)	12/12 (100)
hTERT	Hs00162669_m1	9/9 (100)	9/12 (75)
WT-1	Hs00240913_m1	5/9 (56)	9/12 (75)
VEGFR2	Hs00911700_m1	5/9 (56)	11/12 (92)
VEGFR1	Hs01052961_m1	6/9 (67)	12/12 (100)
Survivin	Hs00153353_m1	9/9 (100)	12/12 (100)

that the responses to these peptides were not specific to T cells from patients with pancreatic adenocarcinoma (Fig. 1b). In other words, peptides $ART1_{188}$, $MAGE\text{-}A3_{195}$, $p53_{161}$, $hTERT_{461}$, $WT\text{-}1_{235}$, and $VEGFR2_{169}$ have specific immunogenic potential in patients with pancreatic adenocarcinoma.

The number of peptide-specific IFN-γ-producing T cells was counted to examine the frequency of T cells responsive to TAA-derived peptides. A range of 10–46 T cells per 300,000 PBMCs in patients with pancreatic adenocarcinoma produced IFN-γ (Fig. 1c).

TAA-specific CTL induction and cytotoxic activity

We attempted to induce peptides specific to CTLs from the PBMCs of pancreatic adenocarcinoma patients. Cytotoxicity assays were performed in more than five patients for each peptide. Of the 11 peptides recognized in more than two patients in the IFN-γ ELISPOT assay, 6 peptides ($MAGE\text{-}A3_{195}$, $p53_{161}$, $hTERT_{461}$, $hTERT_{324}$, $WT\text{-}1_{235}$, and $VEGFR2_{169}$) could induce their specific CTLs, which were confirmed to be able to respond to C1RA24 cells pulsed with corresponding peptides by the cytotoxicity assay, as shown in Fig. 2a.

We conducted a cytotoxicity assay to determine whether peptide-specific CTLs from healthy volunteers could show their cytotoxic activity against pancreatic carcinoma cell lines. $P53_{161}$-, $hTERT_{461}$-, and $hTERT_{324}$-specific CTLs showed cytotoxicity against YPK-2 (HLA-A24-, p53-, and hTERT-positive), but not against Panc-1 (HLA-A24-negative, p53- and hTERT-positive). $MAGE\text{-}A3_{195}$-, $WT\text{-}1_{235}$-, and $VEGFR2_{169}$-specific CTLs also showed cytotoxic activity against YPK-2 (HLA-A24-, MAGE-A3-, WT-1-, and VEGFR2-positive), but not against YPK-1 (HLA-A24-positive, MAGE-A3-, WT-1-, and VEGFR2-negative). Representative data are shown in Fig. 2b.

Phenotypic analysis of TAA-derived peptides specific to T cells

To analyze the characteristics of TAA-derived peptides specific to T cells and select the appropriate epitope for immunotherapy in patients with pancreatic adenocarcinoma, we performed phenotypic analysis by tetramer staining and FACS analysis. We first attempted to detect $MAGE\text{-}A3_{195}$-, $hTERT_{461}$-, and $WT\text{-}1_{235}$-specific tetramer-positive T cells in PBMCs and CTLs induced by the corresponding peptides in healthy volunteers. The ratio of tetramer-positive T cells was increased in CTLs and their frequencies were 1.481–2.930 % of $CD8^+$ T cells, suggesting that these tetramers work well (Fig. 3a). We also conducted similar assays in pancreatic adenocarcinoma patients and detected tetramer-positive T cells in CTLs (Fig. 3b).

We then examined the naïve/effector/memory phenotype of tetramer-positive cells in the PBMCs of patients. The memory phenotype was investigated by the criterion of CD45RA/CCR7 expression [28]. In tetramer analysis, the frequencies of $MAGE\text{-}A3_{195}$-, $hTERT_{461}$-, and $WT\text{-}1_{235}$-specific tetramer-positive T cells were 0.003–0.044,

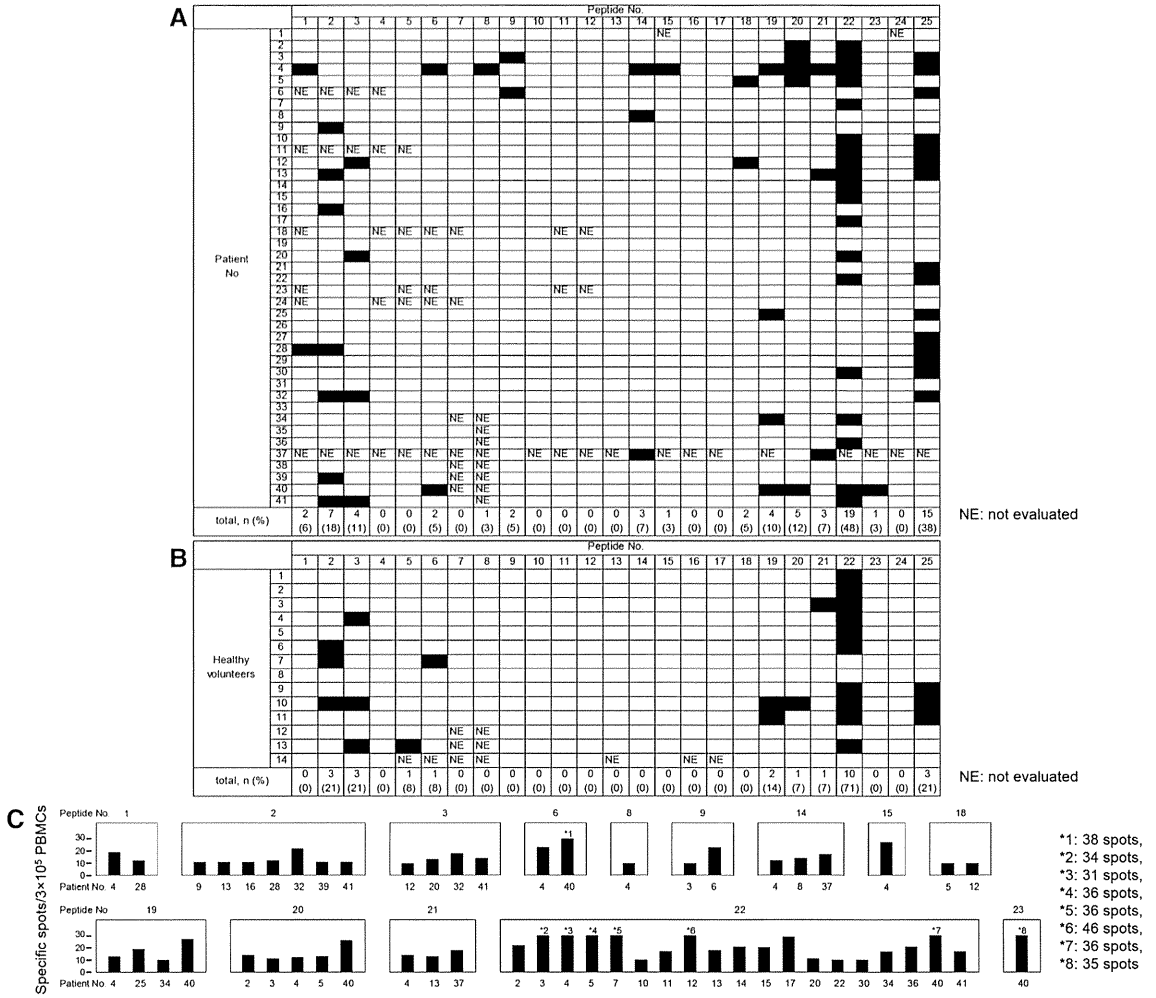

Fig. 1 T cell responses to TAA-derived peptides and control peptides in pancreatic adenocarcinoma patients **a** and healthy volunteers **b**. T cell responses were evaluated by the IFN-γ ELISPOT assay. Responses were considered positive if 10 or more specific spots were detected and if the number of spots in the presence of an antigen was at least twofold that in its absence. *Black boxes* indicate positive responses. **c** The frequency of TAA-specific IFN-γ-producing T cells evaluated by the ELISPOT assay. *Black bars* indicate the response of one patient

0.006–0.053, and 0.030–0.191 % of $CD8^+$ T cells, respectively. The frequency of $CD45RA^-/CCR7^+$ (central memory), $CD45RA^-/CCR7^-$ (effector memory), and $CD45RA^+/CCR7^-$ (effector) T cells in tetramer-positive cells depended on the patient and all phenotypes were observed in all patients, except for patients 1, 8, 28, 29, and 4 (Supplementary Fig. 1).

TAA-specific T cell responses and clinical features of pancreatic cancer patients

In the present study, we analyzed the clinical features that can affect TAA-specific immune responses. When we divided patients into two groups based on their frequencies of lymphocyte subsets in peripheral leukocytes (<24 %, the median value among all patients, or equal to or more than 24 %) and the strength of TAA-specific immune responses into three groups according to the frequency of TAA-specific T cells (<10 specific spots on ELISPOT assays, no response; 10–19 specific spots, weak response; equal to or more than 20 specific spots, strong response), the patients with more lymphocyte subsets in peripheral leukocytes showed stronger TAA-specific T cell responses (Supplementary Fig. 2). On the other hand, we could not find any relationship between TAA-specific immune responses and other clinical characteristics such as age, sex, tumor marker levels, UICC stage, or metastasis status.

We also analyzed the correlation between T cell responses and the prognosis of pancreatic cancer patients. The median overall survival time of patients with T cell responses to at least one TAA-derived peptide evaluated

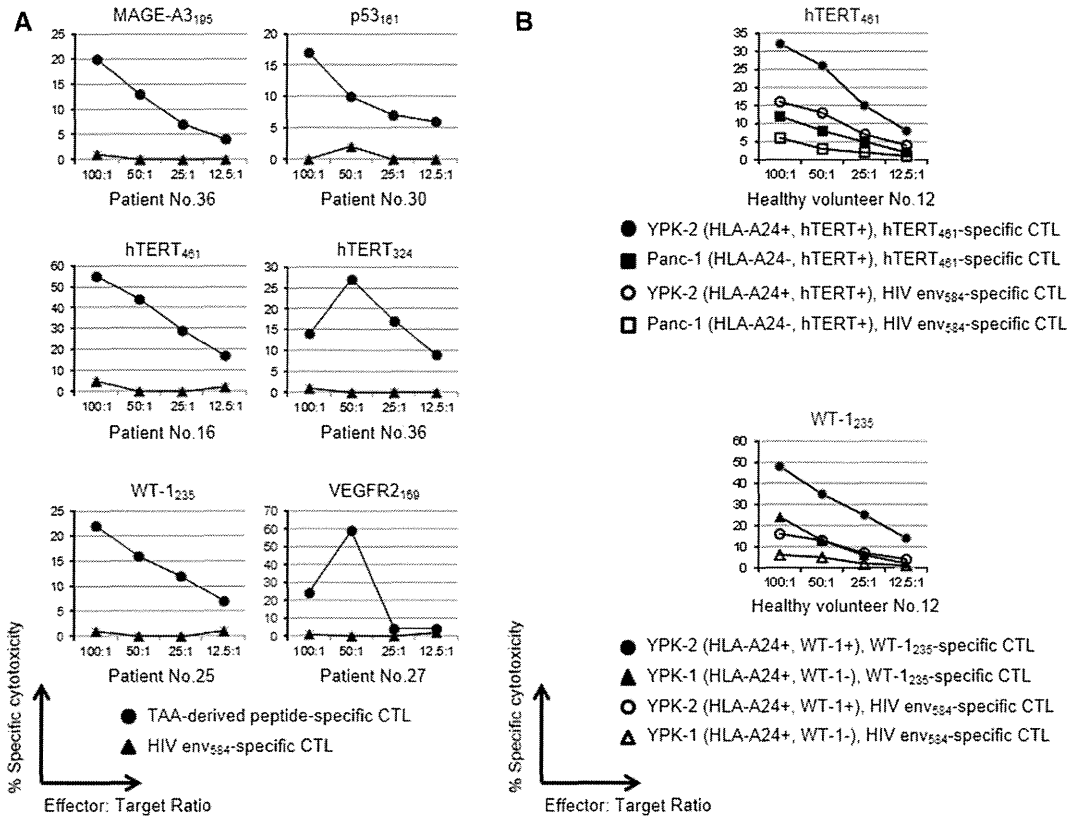

Fig. 2 **a** T cell responses to peptides evaluated by the cytotoxicity assay. Peptide-specific CTL induction and cytotoxicity assays were performed on the PBMCs from at least five patients, and representative data are shown when peptide-specific CTLs were induced in one or more patients. A percent-specific cytotoxicity of more than 10 % was considered to be positive. Six peptides: 9, 14, 18, 19, 20, and 21, could induce their specific CTLs, and these could respond to C1RA24 cells pulsed with the corresponding peptides in the cytotoxicity assay. **b** Cytotoxic activity against the pancreatic carcinoma cell lines of TAA-specific CTLs from healthy volunteers evaluated by the cytotoxicity assay. Cytotoxicity was stronger against pancreatic carcinoma cells that were HLA-A24-restricted and expressed corresponding TAAs than against those not HLA-A24-restricted or not expressing corresponding TAAs

by the ELISPOT assay was 12.2 months, which was significantly longer than that without T cell responses (4.3 months) ($p = 0.013$) (Fig. 4a). On the other hand, no correlation was observed between positive T cell responses and CMV-derived peptides and clinical outcomes (Fig. 4b), suggesting that TAA-specific T cell responses, but not the general immune response, is a prognostic factor in patients with pancreatic adenocarcinoma. The frequencies of regulatory T cells or the ratio of regulatory T cells to $CD8^+$ T cells had no impact on the outcomes of patients in this study.

Discussion

Immunotherapy is considered to be a fourth treatment procedure for cancer following surgical resection, radiotherapy, and chemotherapy [29]. Cancer vaccine therapy was previously shown to convey survival benefits to prostate cancer patients in a clinical phase III trial [30], and some candidates of other cancers have been identified and separately evaluated to determine whether a CTL response can be elicited, with the subsequent elimination of cancer cells and improvement in outcomes. Although a successful clinical response depends on how much tumor antigens elicit their specific CTLs, which are the most important effector cells for antitumor immune responses, to the best of our knowledge, no studies have attempted to identify which epitopes are optimal for peptide vaccine therapy in patients with pancreatic adenocarcinoma. Therefore, we simultaneously compared peptide-specific T cell responses among various TAAs in 41 identical patients with pancreatic adenocarcinoma under the same experimental conditions.

Therapeutic function is the most important factor to consider when determining the usefulness of cancer antigens for peptide vaccine therapy. However, it is very difficult to compare the efficacy of more than one epitope, especially in patients with pancreatic adenocarcinoma whose survival time is very short. Under such circumstances, immunogenicity, specificity, oncogenicity, expression levels, % of

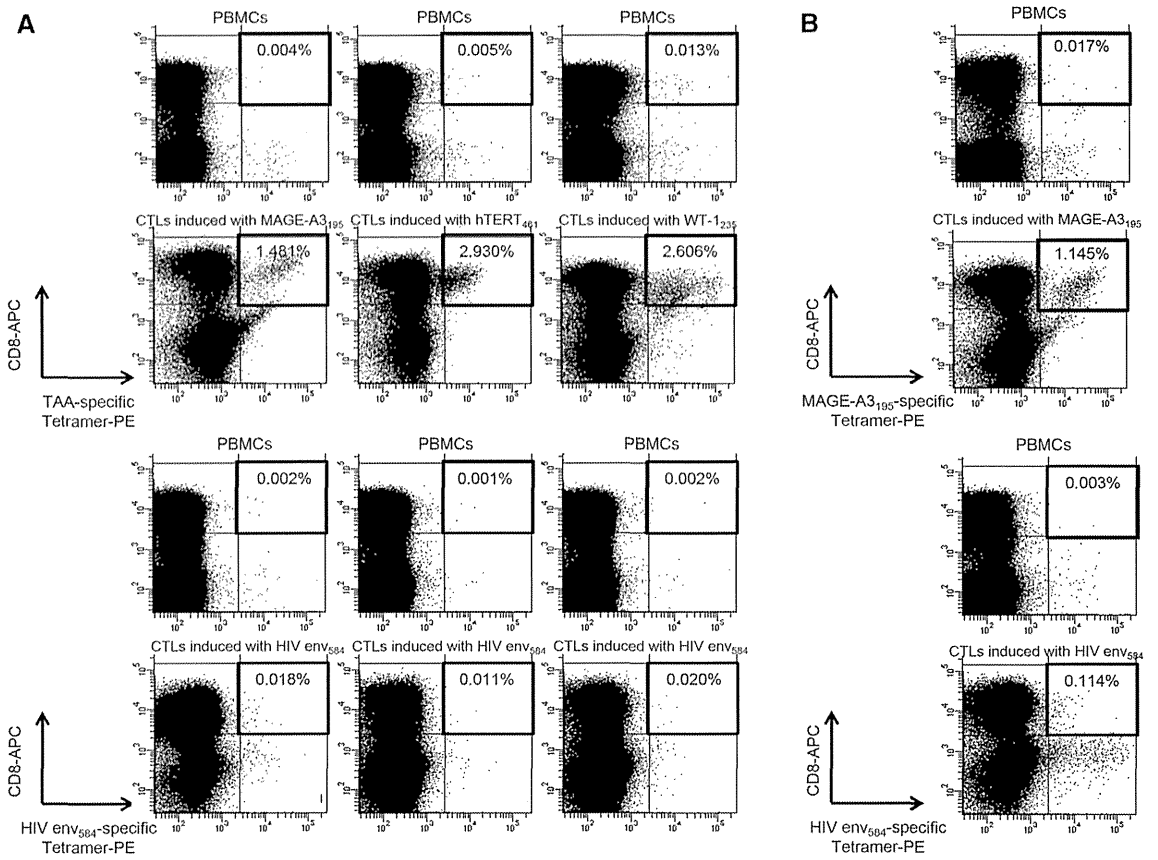

Fig. 3 Detection of TAA-specific, HLA-A24-tetramer$^+$, and CD8$^+$ lymphocytes in PBMCs from healthy volunteers and pancreatic adenocarcinoma patients. **a** Tetramer analyses were performed on eight healthy volunteers for each peptide (MAGE-A3$_{195}$, hTERT$_{461}$, and WT-1$_{235}$). Tetramer$^+$ and CD8$^+$ T cells were detectable in both PBMCs and CTLs induced by their corresponding peptides in at least one healthy volunteer, and representative data are shown in cases in which the ratio of tetramer$^+$ and CD8$^+$ T cells to CD8$^+$ T cells was higher in CTLs induced with each TAA-derived peptide than in PBMCs. **b** Tetramer analyses were performed on pancreatic adenocarcinoma patients using PBMCs and CTLs, which were induced with TAA-derived peptides and showed cytotoxicity against pancreatic cancer cell lines in cytotoxicity assay. Levels of tetramer$^+$ and CD8$^+$ T cells were higher in CTLs induced with TAA-derived peptides than in PBMCs. Representative data are shown in cases in which the ratio of tetramer$^+$ and CD8$^+$ T cells to CD8$^+$ was 0.017 % in PBMCs and 1.145 % in MAGE-A3$_{195}$-specific CTLs

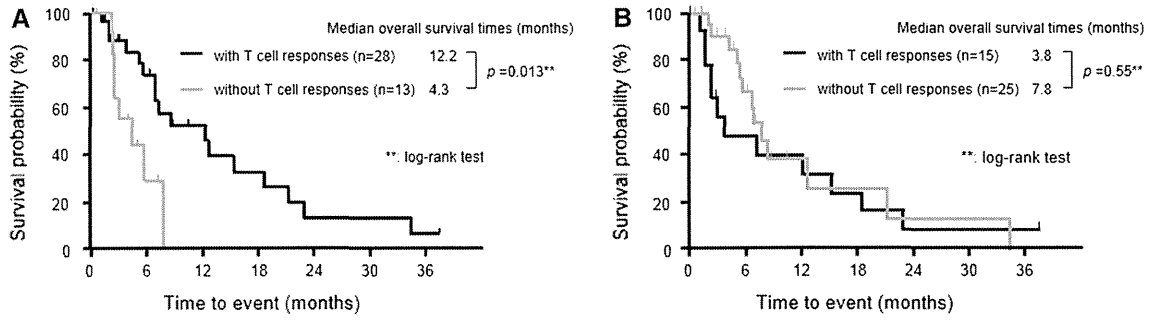

Fig. 4 Kaplan-Meier plot of the overall survival of pancreatic cancer patients according to **a** TAA-specific T cell responses and **b** T cell responses to CMV-derived peptides. **a** TAA-specific T cell responses were defined as positive if 10 or more specific spots to at least one TAA-derived peptide were detected on the ELISPOT assay. The overall survival time of patients with TAA-specific T cell responses was significantly longer than that of patients without TAA-specific T cell responses. **b** T cell responses to CMV-derived peptides were defined as positive if 10 or more specific spots to CMV-derived peptides were detected on ELISPOT assays. No correlation was observed between positive T cell responses to CMV-derived peptides and the clinical outcomes of patients

positive cells, and the number of patients with antigen-positive cancer are considered to be alternative criteria [31]. On the basis of our results, $p53_{161}$, $hTERT_{461}$, $WT\text{-}1_{235}$, and $VEGFR2_{169}$ are considered the most optimal epitopes that satisfy all of the above criteria for peptide vaccine therapy in pancreatic adenocarcinoma patients. Although $MAGE\text{-}A3_{195}$ showed immunogenicity, its expression did not appear to be high in pancreatic adenocarcinoma tissue [32]. Therefore, it may be a candidate for cancer vaccine therapy when MAGE-A3 is confirmed to be overexpressed in pancreatic cancer tissue.

A mutation in the p53 gene and overexpression of the p53 protein have been reported previously in pancreatic adenocarcinoma [33], and all pancreatic cancer cell lines and specimens used in our study expressed p53. Some strategies targeting p53 have been proposed over the last decade [34]. As peptide vaccine therapy, the wild-type p53 peptide is well preserved in mutant p53 because most mutations in the p53 gene are missense mutations, and are considered to be one of the attractive targets as a cancer antigen. The frequencies of the CTL response against HLA-A24-restricted $p53_{161}$ investigated by the ELISPOT assay in head and neck carcinoma and hepatocellular carcinoma were shown to be 35 and 10 %, respectively [35, 36]. Although the frequency of 7 % in our study is lower, given the difference according to the primary tumor site or balance between sensitivity and specificity, induced CTLs showed cytotoxic activity against pancreatic adenocarcinoma cell lines, which suggested that p53 may be an attractive target in patients with pancreatic cancer.

hTERT is widely overexpressed in various cancer cells including pancreatic cancer [37], which is consistent with our results. A clinical trial demonstrated that GV1001, a HLA class II epitope corresponding to the hTERT (611–626) fragment, was immunogenic in pancreatic cancer patients [38]. Another previous study evaluating T cell responses to several hTERT epitopes in patients with hepatocellular carcinoma [39] demonstrated that $hTERT_{461}$- and $hTERT_{324}$-specific CTLs were induced in 5 (6.9 %) and 9 (12.5 %) of 72 patients, respectively. In the current study, these frequencies were equivalent and the killing of pancreatic cancer cell lines was demonstrated, which suggested that these epitopes also had immunogenicity in pancreatic cancer patients.

Peptide vaccine therapies using $WT\text{-}1_{235}$ and $VEGFR2_{169}$ combined with gemcitabine have already been conducted in pancreatic adenocarcinoma patients [23, 24]. We clarified that $WT\text{-}1_{235}$- and $VEGFR2_{169}$-specific CTLs induced from PBMCs showed cytotoxicity for human pancreatic cancer cell lines, and the results of further investigations are anticipated.

We performed phenotypic analysis of TAA-derived epitope-specific T cells to determine the most appropriate epitope for immunotherapy in patients with pancreatic adenocarcinoma. Epitope-specific tetramer$^+$ cells in PBMCs were also found in patients without IFN-γ ELISPOT responses, which was consistent with the findings of previous studies [39, 40] and suggested the existence of dysfunctional epitope-specific T cells. Epitope-specific tetramer$^+$ cells were also identified at a very low frequency in PBMCs from healthy volunteers and increased in CTLs induced with TAA-derived peptides, which was also consistent with previous studies in which TAA-specific tetramer$^+$ T cells were detectable in samples from healthy donors [41] or the in vitro stimulation of PBMCs with the epitopes derived from TAA could induce TAA-specific CTLs in healthy volunteers [42], even though the precise mechanism has not yet been clarified. Phenotypic analysis showed that the frequency of T cells with each memory and effector phenotype depended on the patient and also that peptide-specific memory T cells existed in PBMCs of patients with pancreatic adenocarcinoma. Because T cells with the memory phenotype exert stronger antitumor effects by secondary stimulation with the antigen, our results suggest that an additional immunological approach such as that consisting of a TAA-derived protein or peptide, recombinant virus, and engineered tumor cells to boost T cell function may be useful to enhance host antitumor immune responses.

Another purpose of this study was to identify the factors influencing immune responses. Our results suggested that the frequencies of the lymphocyte subsets in peripheral leukocytes were very important in the induction of TAA-specific CTLs. Although the relationship between cancer, inflammation, and immunity has already been documented [43], the precise mechanism has yet to be fully understood. One of the speculated reasons why PBMC from patients with lymphocytopenia could not induce a good immune response in our study is that the release of inhibitory immunological cytokines such as transforming growth factor β or IL-10 from pancreatic adenocarcinoma tissue decreases lymphocyte counts and impairs the function of lymphocytes both systemically and in the microenvironment [44]. It was also reported that lymphocyte counts and CTL responses were prognostic markers in advanced cancer cases receiving peptide vaccine therapy [45, 46]. Our results showing a correlation between the T cell response and outcomes in pancreatic adenocarcinoma patients corresponded to these previous findings, which indicate that restricting the objective to those with an adequate lymphocyte subset could lead to a clinical trial with favorable outcomes.

A limitation of this study was the lack of data for the clinical response. Tumor shrinkage or survival benefits are not always observed in all patients who exhibit immune responses. Further, clinical studies using peptides that could induce TAA-specific CTLs are needed to confirm our findings.

In conclusion, we simultaneously compared T cell responses to various TAA-derived epitopes in patients with pancreatic adenocarcinomas; our results suggested that $p53_{161}$, $hTERT_{461}$, $WT\text{-}1_{235}$, and $VEGFR2_{169}$ were the most suitable epitopes for cancer vaccine therapy.

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Conflict of interest The authors do not have any conflict of interest.

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PHASE I STUDIES

Phase I dose-escalation study evaluating safety, tolerability and pharmacokinetics of MEDI-573, a dual IGF-I/II neutralizing antibody, in Japanese patients with advanced solid tumours

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Summary *Purpose* This Phase I, open-label, single-arm, dose-escalation study aimed to evaluate the safety and tolerability of the insulin-like growth factor (IGF-I/II) neutralizing antibody, MEDI-573, in Japanese patients with advanced solid tumours refractory to standard therapy or for which no standard therapy exists. The pharmacokinetics, pharmacodynamics and antitumour activity of MEDI-573 were also evaluated. *Methods* Three cohorts of patients received MEDI-573 in escalating order: cohort 1, 5 mg/kg on Day 1, 8 and 15; cohort 2, 15 mg/kg on Day 1, 8 and 15; cohort 3, 45 mg/kg on Day 1, of 21-day cycles. *Results* Ten patients who received at least one dose of MEDI-573 were evaluated. The median number of treatment cycles was 2.0 (range 1–6) and the median number of MEDI-573 doses received was 4.0 (range 1–17). The most commonly reported drug-related adverse events were fatigue (n=2 patients), pyrexia (n=2), diarrhoea (n=2) and electrocardiogram QT prolongation (n=2). No patients experienced a dose-limiting toxicity. Pharmacokinetics of MEDI-573 were linear with a dose-dependent increase. There were no complete or partial responses; four patients had an overall best response of stable disease. *Conclusions* MEDI-573 is well tolerated at the doses investigated.

Keywords Novel antitumour agent · IGF-II/I inhibitor · MEDI-573 · Monoclonal antibody · Phase I · Solid tumours

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Introduction

The complex insulin-like growth factor (IGF) system plays a critical role in normal cell growth and development [1, 2] and may represent an important target for the development of antitumour therapies. The IGF signalling pathway consists of circulating stimulatory ligands (IGF-I and IGF-II), cell surface receptors (the type I IGF receptor [IGF-IR] and the type II IGF receptor [IGF-IIR]), and IGF binding proteins [3, 4]. Inhibition of IGF signalling has been shown to enhance the response of tumour cells to chemotherapy [5, 6] while down-regulation of IGF-IR expression and/or inhibition of signalling leads to inhibition of tumour growth, both in vitro and in vivo, most notably in Ewing's sarcoma [7].

MEDI-573 is a fully human immunoglobulin G2 lambda monoclonal antibody generated by Xenomouse® (Abgenix) technology that selectively binds to human IGF-II and IGF-I [6]. Preclinical evaluation of MEDI-573 has demonstrated inhibition of IGF-induced signalling pathways and cell proliferation in cancer cell lines, and inhibition of in vivo growth in tumour xenografts [6].

This Phase I, open-label, single-arm, dose-escalation, study (NCT01340040) aimed to evaluate the safety and tolerability of MEDI-573 in Japanese patients with advanced solid tumours. Secondary objectives included assessment of pharmacokinetics (PK), pharmacodynamics (PD), immunogenicity and antitumour activity.

Methods

Patients

Eligible patients were Japanese men or women aged ≥20 years with histologically/cytologically-confirmed advanced solid tumours refractory to standard therapy or for which there

was no standard therapy available; World Health Organization performance status of 0 to 2; life expectancy of ≥4 months; and ≥1 measurable and/or non-measurable lesion (refer to supplementary appendix for exclusion criteria).

Study design and intervention

This Phase I, open-label, single-arm, dose-escalation study (clinicaltrials.gov identifier NCT01340040) was conducted in one centre (Shikoku Cancer Center) in Japan. This study was carried out in accordance with the Declaration of Helsinki and International Conference on Harmonisation Good Clinical Practice guidelines and AstraZeneca policy on bioethics. The study protocol was approved by the responsible ethics committee. All patients provided informed consent.

Three cohorts (cohort 1: MEDI-573 5 mg/kg administration on Day 1, 8 and 15; cohort 2: MEDI-573 15 mg/kg administration on Day 1, 8 and 15; cohort 3: MEDI-573 45 mg/kg administration on Day 1, of 21-day cycles) were studied in escalating order. Patients were considered dose-limiting toxicity (DLT)-evaluable when they had either received three doses of MEDI-573 (cohorts 1 or 2) or one dose of MEDI-573 (cohort 3) during the first cycle or, alternatively, had experienced a DLT during the cycle (refer to supplementary appendix for further DLT information).

MEDI-573 was administered once every 7 days (on Day 1, 8, and 15 of each 21-day treatment cycle) as a 60-min intravenous (IV) infusion in cohorts 1 and 2 and once every 21 days (Day 1 of each 21-day treatment cycle) as a 90-min IV infusion in cohort 3 until unacceptable toxicity, documentation of disease progression or other reasons for subject withdrawal. The dosing interval was 6 days or longer from cycle 2 onwards in cohorts 1 and 2, and 20 days or longer in cohort 3. Intra-subject dose escalation was not allowed.

Dose escalation and cohort size were based upon accepted methodology for Phase I oncology studies, as defined by European and United States regulations. Based primarily on previous results of a Phase I dose-escalation study of MEDI-573 in predominantly Western (white) patients with advanced solid tumours [8], the starting dose of MEDI-573 used in the current study was 5.0 mg/kg/week. The regimen for cohort 3 (administration every 21 days) was selected based on the results of cohort 2 in this study and results from the previous dose-escalation study of MEDI-573.

Objectives

The primary objective was to evaluate the safety and tolerability of MEDI-573 in Japanese patients with advanced solid tumours refractory to standard therapy or for which no standard therapy exists. Secondary objectives were: to assess PK and immunogenicity of MEDI-573; to measure PD of MEDI-573 on circulating plasma levels of IGF-I and IGF-II; to observe any antitumour activity of MEDI-573; and to determine the maximum tolerated dose (MTD) of MEDI-573.

Assessments

Safety

Adverse events (AEs) were graded according to the National Cancer Institute CTCAE, version 4.0. AEs and serious adverse events (SAEs) were assessed throughout the study and for 30 days following the last dose of MEDI-573. Similarly, vital signs and laboratory parameters were assessed throughout the study and for 30 days following the last dose of treatment.

Pharmacokinetics, pharmacodynamics and immunogenicity

Blood samples for assessment of PK parameters and PD parameters (free and bound IGF-I and IGF-II) were collected on Day 1, 2, 3, 8 and 15 of cycle 1 and Day 1, 8, and 15 of cycle 2, at the end of treatment, and 30 days after the last dose of treatment. The quantitative determination of free IGF-I & IGF-II in human plasma was conducted using two validated direct-binding electrochemiluminescence (ECL) assays based on the Meso Scale Discovery® (MSD; Rockville, USA) detection platform. The working ranges of the free IGF-I and IGF-II assays for 100 % heparinized plasma were 0.313 ng/mL — 50.000 ng/mL inclusive and 0.625 ng/mL and 20.000 ng/mL inclusive, respectively. PK parameters were assessed by a non-compartmental approach using WinNonlin software (Pharsight Corporation, Cary, NC, USA).

For determination of free MEDI-573 and assessment of immunogenicity, please refer to the supplementary appendix.

Blood samples for assessment of anti-MEDI-573 antibodies were collected on Day 1 of cycle 1 and cycle 2, and 30 days and 3 months after the last dose of treatment.

Response

Tumour assessment was performed according to response evaluation criteria in solid tumours (RECIST), version 1.1, ≤28 days before the start of study treatment, on Day 1 of cycle 2 and until discontinuation of study treatment. Patients who discontinued study treatment for reasons other than disease progression or initiation of alternative anticancer therapy, underwent tumour assessment 3 months after the last dose of MEDI-573.

Statistics

Data were presented descriptively for safety, PK, PD, immunogenicity and efficacy. All patients who received ≥1 dose of MEDI-573 were included in the safety analysis set. All

Table 1 Patient demographics and baseline characteristics

	MEDI-573		
	5 mg/kg (n=4)	15 mg/kg (n=3)	45 mg/kg (n=3)
Age, median (range)	60.0 (55–68)	68.0 (47–70)	58.0 (52–72)
Sex			
Male, *n*	0	2	1
Female, *n*	4	1	2
Tumour type			
Colorectal	0	0	1
Colon	1	1	0
NSCLC	1	1	2
Pancreas	2	1	0
Stage IV disease	4	3	3
Number of prior cancer treatments, median (range)	8.5 (5–12)	8.0 (5–8)	8.0 (6–8)
Prior cancer treatments[a]			
Biological	2	1	1
Chemotherapy	4	3	3
Radiation	0	0	2
Surgery	3	1	2
Other	2	3	3
Best response to the last prior cancer treatment			
CR/PR/SD/PD	0/4/0/0	0/2/1/0	0/2/1/0

[a] Patients with multiple prior treatments in the same category were counted only once in that category. Patients with prior treatments in more than one category were counted once in each of those categories

CR, complete response; NSCLC, non-small-cell lung cancer; PD, progressive disease; PR, partial response; SD, stable disease

patients who received ≥1 dose of MEDI-573 and for whom an adequate PK profile had been obtained, were included in the PK analysis set. All patients who received ≥1 dose of MEDI-573 with any post-PD data were included in the PD analysis set. The MTD dataset included all patients who had received ≥1 dose of MEDI-573 and completed the safety follow up through the DLT evaluation period, or who experienced a DLT.

Results

Patients

Between July 2011 and May 2012, 12 patients were enrolled in this study at one centre in Japan; of these, 10 patients received MEDI-573 (all were Japanese; two patients did not meet inclusion criteria) and were evaluable for safety and efficacy analyses. Patient demographics and baseline characteristics are summarized in Table 1. Mean age was 61.0 years (range 47–72); three were male and seven female. Two patients had colon cancer, one had colorectal cancer, four had non-small-cell lung cancer and three had pancreatic cancer. The median number of prior cancer treatments was 8.0 (range 5–12), and all 10 patients had received prior chemotherapy.

Four patients were included in the 5 mg/kg group, three in the 15 mg/kg group and three in the 45 mg/kg group. Eight

Table 2 Adverse events reported in ≥2 patients overall, by preferred term

	Number of patients[a]		
Adverse event	MEDI-573 5 mg/kg (n=4)	MEDI-573 15 mg/kg (n=3)	MEDI-573 45 mg/kg (n=3)
Pyrexia	2	1	0
Diarrhoea	2	0	0
Vomiting	2	0	0
Constipation	1	1	0
Fatigue	0	1	1
Electrocardiogram QT prolonged	0	1	1
Anaemia	1	0	1
Tinnitus	1	0	1
Insomnia	1	0	1

[a] A patient experiencing more than one adverse event within a preferred term was counted only once