厚生労働科学研究費補助金（ヒトゲノム・再生医療等研究事業）
分担研究報告書

骨髄低形成を呈した非寛解期 AML/RAEB に対する骨髄非破壊的前処置による臍帯血移植

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研究要旨

初回治療抵抗性を示す高齢者 AML/RAEB の治療成績は極めて不良である。初回寛解導入療法後に芽球が残存するものの骨髄が低形成となり、汎血球減少症を呈した３症例に対し、HLA 一致血縁ドナー不在のため臍帯血を用いた迅速な低強度造血幹細胞移植(reduced intensity stem cell transplantation:RIST)を行った。年齢は５８歳から６７歳。３例とも強力な寛解導入療法の後寛解には至らず、８－３０％の芽球が残存した骨髄低形成となった。不応性および HLA 一致血縁ドナー不在確認後、寛解導入療法開始日から RIST までは６０－１０３日と速やかに臍帯血 RIST を行った。前処置は fludarabine 125－150 mg/m^2 + melphalan　80－140 mg/m^2 + TBI 4Gy で、１例は ALG を併用した。生着は全例に確認され、grade Ⅱの急性 GVHD を２例に認めた。１例で移植後早期に原因不明の脳炎を合併した。３例とも長期に持続する CMV 抗原血症を認め、移植後半年以上経過した後、B 型肝炎の増悪、水痘、重症単純疱疹の合併を認めた。臍帯血 RIST は骨髄低形成を呈した非寛解 AML/RAEB に有効な救援療法と考えられるが、ウイルス感染に注意を払う必要がある。

A. 研究目的

初回の寛解導入療法に不応性となった AML/RAEB の予後は極めて不良である。不応性 AML/RAEB に対して唯一治癒が期待される方法は同種造血幹細胞移植(allogeneic stem cell transplantation:allo-HSCT)である。しかし、高齢者では通常の前処置が行えないため、高齢者の不応性 AML/RAEB に対する有効な治療法は存在しなかった。近年、低強度造血幹細胞移植(reduced-intensity stem cell transplantation:RIST)と臍帯血移植が普及し、allo-HSCT の適応が年齢や合併症のバリアを超えて拡大しつつある。また臍帯血は HLA 一致ドナーを見つけにくい高齢者にとって重要な幹細胞源であり、さらに非血縁骨髄に比べて短期間に入手できる利点がある。そのため高齢者の不応性 AML/RAEB に対して行い得る allo-HSCT として臍帯血 RIST が注目されている。

非寛解または不応性 AML/RAEB に対し allo-HSCH を行う際に、移植前の骨髄中の白血病細胞量が再発の危険因子になることが知られている(Sierra et al, Blood,1997)。特に RIST では前処置の抗腫瘍効果が弱いため、通

常の前処置のallo-HSCTに比べ再発が高率におこることが予測される。今回、腫瘍量の少ない骨髄低形成となった不応性AML/RAEBを対象に臍帯血RISTの有用性を検討した。

B. 研究方法

AMLまたはRAEBでanthracyclineを含む寛解導入療法後、芽球の残存を認めるものの骨髄低形成となった５５歳以上の症例で、HLA一致血縁ドナーが不在である症例を対象とし、臍帯血RISTの効果と安全性を検討した。

C.研究結果

【症例１】65歳男性AML(M0)。寛解導入療法(DNR4+BHAC10)後、芽球の残存(10%)、骨髄低形成および汎血球減少を認め、緑膿菌敗血症を合併した。敗血症治癒後の寛解導入療法開始日から９７日目にfludarabine 125 mg/m^2+ L-PAM 100 mg/m^2+TBI 4Gyによる臍帯血移植を行った。生着は速やかに確認された（好中球１４日目、血小板４２日目)。移植後１４日目頃より原因不明の脳炎を発症、一時呼吸管理を要したが、認知症を残すものの治癒した。Ⅱ度の急性GVHDを認めたがステロイド治療にて軽快した。移植後１５日目から１２７日目までサイトメガロウイルス(CMV)抗原血症とB型肝炎の増悪を合併したが軽快し、７６０日を超え無再発生存中である。【症例２】57歳女性。治療関連RAEB-Ⅱを発症。2回の寛解導入療法(IDR3+AraC7など)後、寛解には至らず芽球の残存(8%)、骨髄低形成および汎血球減少を認めた。寛解導入療法開始日より１０３日目にfludarabine 125 mg/m^2+L-PAM 140 mg/m^2+TBI 4Gyによる前処置後に臍帯血移植を行った。生着は速やかに確認された（好中球１９日目、血小板３９日目)。Ⅱ度の急性GVHDを認めたがステロイド治療で軽快した。移植後４２日目から１３４日目までCMV抗原血症を認めたが軽快し、７３０日を超え無再発生存中である。【症例３】67歳女性。MDSから移行したAML。寛解導入療法(DNR3+BHAC12)後寛解に至らず芽球の残存(30%)、骨髄低形成および汎血球減少を認めた。Fludarabine 150 mg/m^2+L-PAM 80 mg/m^2+TBI 4Gyによる前処置後臍帯血移植を施行した。好中球の生着は１８日目に認められたが、血小板は９８日目に5x10^4/ml以上となった。GVHDの合併はなかったが、移植後４０日目から５６日目までCMV抗原血症と単純疱疹の合併を認めた。移植後２５０日を超えて無再発生存中である。

D.考察

芽球比率の高い不応性・再発性AML/RAEBに対するallo-HSCTでは、移植後の再発が問題となる。今回、骨髄低形成となった非寛解期のAML/RAEBを対象に臍帯血RISTを行ったところ、良好な移植成績が得られた。高齢者に対するallo-HSCTではRISTが限界と考えられる。RISTでは抗腫瘍効果が弱い点を考えると対象は腫瘍量が少ない症例に限られる。また、幹細胞ソースに臍帯血を用いれば、感染症などの合併症を起こす前に迅速に移植することが出来る。今回の

結果より、非寛解例であっても骨髄が低形成であれば臍帯血 RIST が有効であることが示唆された。しかし、3例とも複数のウイルス感染症を合併していることから、感染症に対する対策を十分に講じる必要があると思われた。

E. 結論

骨髄が低形成の再発・不応性 AML/RAEB に対しては臍帯血 RIST が有効である可能性が示唆された。多数例で検討する必要がある。

F. 健康危険情報

なし

G. 研究発表

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H. 知的財産権の出願・登録状況

1) 特許取得　なし

2) 実用新案登録　なし

3) その他　なし

厚生科学研究補助金（ヒトゲノム・再生医療等研究事業）
分担研究報告書

HLA 一致血縁ドナー以外のドナーから同種造血幹細胞移植の開発

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研究要旨
HLA 一致血縁ドナーが存在せず、年齢および合併症などにより従来型の造血幹細胞移植が適応とならない患者に対する新規造血幹細胞移植療法の開発を行った。HLA 血清型完全一致非血縁ドナーから採取された骨髄幹細胞を使用し、従来の前処置療法と比較して治療強度を軽減した同種造血幹細胞移植（reduced-intensity stem cell transplantation; RIST）いわゆるミニ移植術を行い、その有効性と安全性を評価することを目的とする臨床試験を遂行した。2006 年 2 月現在で、目標症例 25 例に対して 26 症例の登録を終え、本試験を終了した。

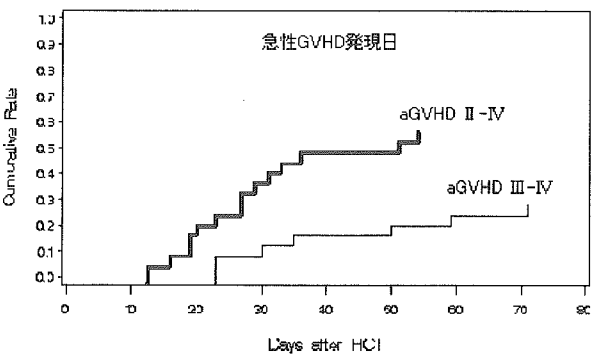
A. 研究目的

HLA 一致血縁ドナーからの同種造血幹細胞移植は、造血器疾患に対する根治的な治療法として確立している。HLA が一致し適格な血縁ドナーがいない場合、幹細胞ソースとして、HLA 不一致血縁ドナー、非血縁 HLA 一致ドナー、非血縁臍帯血などが、考慮される。さらに、従来型の造血幹細胞移植は、患者の年齢、および合併症などによりその適応が制限されていた。しかしながら、白血病などの造血器悪性腫瘍は加齢と共にその発症頻度が増加する。そのため、より安全な移植療法の開発が重要である。
本研究では、他に有効な治療法を持たない難治性造血器疾患を有するにもかかわらず、根治療法としての通常の骨髄破壊的移植術が適応とはならない患者を対象とする。HLA 血清型完全一致非血縁ドナーから採取された骨髄幹細胞を使用し、従来の前処置療法と比較して治療強度を軽減した同種造血幹細胞移植（reduced-intensity stem cell transplantation; RIST）いわゆるミニ移植術を行い、その有効性と安全性を評価する。
本研究の特徴は、ミニ移植術で一般に用いられる HLA 一致血縁ドナー由来の末梢血幹細胞を用いずに、HLA 一致非血縁ドナーの骨髄幹細胞を用いた場合の成績を検討することである。

B. 研究方法

本研究では、他に有効な治療法を持たない難治性造血器疾患を有するにもかかわらず、根治療法としての通常の骨髄破壊的移植術が適応とはならない患者を対象とする。HLA 血清型完全一致非血縁ドナーから採取された骨髄幹細胞を使用し、従来の前処置療法と比較して治療強度を軽減した同種造血幹細胞移植（reduced-intensity stem cell transplantation; RIST）いわゆるミニ移植術を行い、その有効性と安全性を評価する。移植前治療は、2-CdA 0.11mg/kg x 6 days, Bu 4mg/kg x 2 days, TBI 4Gy、GVHD 予防は CSP+sMTX で行う。

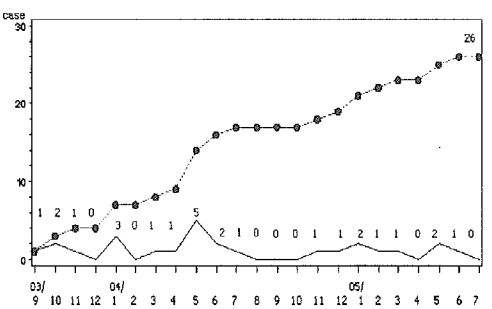
主要評価項目 (primary endpoint)
（1）　移植後 28 日時点での生着
（2）　造血回復までの期間、完全キメラ達成までの期間および割合(移植後 day 90±5 でドナー由来細胞が 90%以上)
副次的評価項目（secondary endpoint）
（1）　移植後 100 日時点の全生存率および無増悪生存率
（2）　移植後 1 年の全生存率および無増悪生存率
（3）　前処置・急性移植片対宿主病(graft-versus-host disease; GVHD)予防薬の



毒性
（4） GVHD の頻度･重症度
（5） 感染症発症率

対象
難治性造血器疾患に罹患し、他の治療では治癒や長期生存が期待できないような病気や病状であるにもかかわらず、年齢制限（50 歳以上）や臓器機能障害、大量化学（放射線）療法の既往があるために通常の血縁/非血縁者間同種造血幹細胞移植の適応にならない患者を本研究の対象とする。血清検査において、HLA の A/B/DR 座の全てが適合した 6/6 完全一致または 5/6 一致の血縁ドナーを有さず、HLA 完全一致血縁ドナーが存在し骨髄提供の同意を得ることができた患者において、説明同意書を用いて同意を得た者について行う。

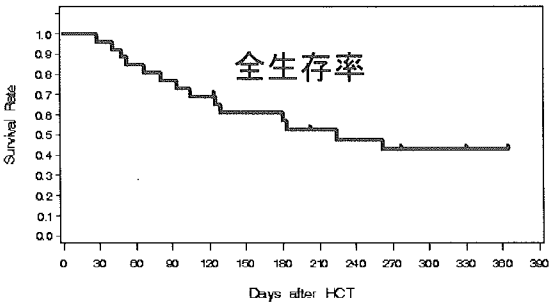
C. 研究結果
2005 年 7 月までに 26 名の症例登録が行われた。年齢は 36－64 才(中央値 56.5 才)、男性 16 人、女性 10 人、登録時の PS は、0　17 人、1　9 人であった。ミニ移植の適応理由は年齢が 73%で最も多かった。疾患は低リスク 65%、ハイリスク　35%であった。

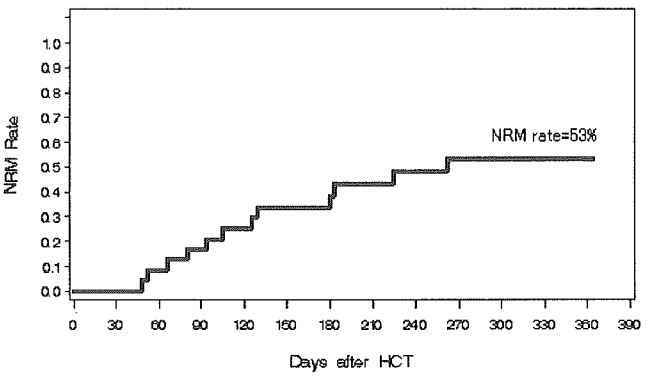


移植関連毒性は、NCI-CTC で grade3 以上が、心臓　4%、肺　16%、肝臓　31%、口腔粘膜 69%、消化管　34%であった。
急性 GVHD は、0　27%、1　15%、2　27%、3　27%、4 4%であった。

慢性 GVHD は、評価可能な 13 名中、限局型 31%、全身型 69%であった。CMV 抗原血症陽性化は、88%、CMV 感染症は 23%に発症した。累積非再発死亡率は 53%であった。
死因は、原疾患の進行 1 名 (7%)、感染症 9 名 (64%)、GVHD + 感染症 4 名 (29%)であった。主要評価項目の day28 までの生着は 25 名(96%)、day90 までの完全キメラ達成は早期死亡した 2 名を除く 24 名全例で行われた。

生存者追跡期間中央値 365 日(124-365) 2005 年 12 月 26 日現在、生存 12 名であった。





D. 考察
本試験は、非血縁ドナーからのミニ移植多施設共同研究である。本試験の結果から、移植前治療 2-CdA 0.11mg/kg x 6 days, Bu 4mg/kg x 2 days, TBI 4Gy により充分な生着が期待できること、CSP+sMTX では急性 GVHD の予防が必ずしも充分ではなく、GVHD を発症した例ではその生命予後が悪化すること、原疾患の進行よりも治療関連死亡の方が多いこと、などが明らかとなった。本治療は充分な抗腫瘍効果を有するため、今後はより強力な GVHD の予防を行うことにその治療成績が改善することが期待される。

E. 結論
□非血縁者間骨髄ミニ移植の多施設共同臨床第 I 相試験を完遂した
□生着、完全キメラといった主要評価項目はクリアした
□原疾患の増悪を来す患者は少なかった
□しかし GVHD や感染症（特に敗血症）をコントロールしきれずに死亡する患者が多かった。

本研究から、HLA 一致非血縁ドナーからミニ

移植は可能であるが、依然として治療関連死亡が多く改良の余地があることが明らかとなった。今後、移植後免疫抑制療法の改善、感染症に対する支持療法の進歩などが望まれる。

G. 研究発表

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H. 知的財産権の出願・登録状況

なし

厚生労働科学研究費補助金（ヒトゲノム・再生医療等研究事業）
分担研究報告書

高齢者 HLA 不一致移植の安全性の検討

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研究要旨
高齢者に対する前処置減弱レジメンを用いた同種骨髄移植 (RIST) では、移植前に評価した併存疾患 (Comorbidity) をスコア化する Comorbidity Index が治療関連死亡の予測因子として有用である。これを用いることにより、移植前処置や移植片対宿主病の予防を個別化、最適化出来る可能性がある。

A. 研究目的

高齢者の多くが合併する併存疾患（comorbidy）を系統的に評価し、スコア化する Hematopoietic stem cell transplantation specific comorbidity index (HCT-CI) が米国のグループによって開発された。これがわが国の前処置減弱レジメンを用いた同種造血幹細胞移植患者にも適用可能であるかを検討し、高齢者 HLA 不一致移植を安全に行うための、治療の個別化、最適化の方法を検討する。

B. 研究方法

国立がんセンター中央病院において、2000 年から 2004 年の 5 年間に前処置減弱レジメンを用いた同種造血幹細胞移植 (Reduced-intensity conditioning allogeneic stem cell stansplantation; RIST) を実施した 160 例について、後方視的に検討した。年齢中央値は 53 歳（幅 15-68 歳）、原疾患は標準リスクが 28 例で、残る 132 例 (83%) は再発のハイリスクであった。ドナーは HLA 一致血縁者が 82 人であり、残る 78 例 (49%) は HLA 不一致血縁者（21 例）、または非血縁者（57 例）であった。移植前処置は全例ブスルファン 8mg/kg が用いられ、フルダラビン 180mg/m2 、またはクラドリビン 0.66mg/kg を併用された。非血縁間移植では更に全身放射線照射 4Gy が用いられた。
移植前処置開始前の併存疾患の状態を診療録の記載から後方視的に調査し、各患者の HCT-CI score (Sorror M. L. et.al Blood 2005 vol.106 No.8 2912 を 算出した。移植後の治療関連死、及び生存期間をエンドポイントとし、HCT-CI を含む各種の因子が治療結果に及ぼす影響について、単変量、多変量解析を用いて解析した。

C. 研究結果

HCT-CI score は 0 点（comorbidity なし）が 55%、1 ないし 2 点が 29%、3 点以上が 16%と、約半数の症例で何らかの comorbidity を有していた。単変量解析にて、移植後 2 年経過時点での累積非再発死亡発生率は HCT-CI 0 点の症例では 18%, 1-2 点では 32%, 3 点以上の患者群では 53% と、HCT-CI score と非再発死亡率の間に有意かつ強い相関を認め（図 1）、これを反映して全生存期間においても HCT-CI score との間に強い相関を認めた（図 2）。
多変量解析においては、非再発死亡においても最も強い予後因子は HCT-CI であり、検討したその他の項目としてはドナーが HLA 一致血縁者以外の場合が有意なリスク因子であった。

D. 考察

当研究班で昨年度報告した通り、高齢者に対する RIST は、HLA allele ミスマッチであったり、移植片が骨髄であるという不利な条件下でも、安定した生着が得られる事が明らかとなっている。RIST による前処置関連毒性は比較的軽度であり、高齢者でも十分に実施可能であると思われたが、非血縁者由来骨髄を用いた RIST では、生着後の非再発死亡が高率であり、その最大の原因は移植片対宿主病が関与したものであった。、今回の検討により、RIST 後の治療関連死亡の危険性は全ての患者において均等でなく、移植前の併存疾患の種類とその重症度に大きく依存していることが判明した。これを評価するために HCT-CI score は極めて有用であり、強力な移植片対悪性腫瘍効果を引き出すことにより、原疾患の再発を予防することが重要な患者群と、移植片対宿主病予防を強化することにより非再発死亡を防ぐ事が重要な患者群を予測することが出来ると考える。すなわち、昨年度の研究成果と合わせ、高齢者 HLA 不一致移植を安全かつ有効な治療法として開発する上で、患者の状態を個別化し、以下の開発方針を取ることが重要であるとの結論に至った。

併存疾患が軽度で疾患リスクが低い患者群：基本的に予後良好な群と考えられるため、移植片の確実な生着が得られる前処置を考慮し、移植片対宿主病も強力に予防するべきである。

併存疾患が重度で、疾患リスクが低い患者群：再発よりも非再発死亡が問題となるため、毒性の低い前処置を用いるとともに、移植片対宿主病予防を強力に行うべきである。

併存疾患が軽度で、疾患リスクが低い患者：再発が最も重要な問題であるため、強力な移植片対宿主病対策は必用とせず、症例によっては前処置療法の強化も考えるべきである。

併存疾患が重度で、疾患リスクが高い場合：安全な移植が可能な適切なドナーが得られない条件下では、現時点では同種造血幹細胞移植によって救命することは困難であり、新たな治療戦略の開発が必用である。

E. 結論

高齢者に対する同種造血幹細胞移植においては、併存疾患を正確に評価し、それに応じた治療方法の個別化が有用な可能性がある。

F. 健康危険情報

該当せず

G. 研究発表

◆ 論文発表

投稿準備中

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H. 知的財産権の出願・登録状況

なし

Ⅲ．　研究成果の刊行に関する一覧

研究成果の刊行に関する一覧表

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Ⅳ. 研究成果の刊行物・別刷

Graft-versus-Tumor Effect Against Advanced Pancreatic Cancer after Allogeneic Reduced-Intensity Stem Cell Transplantation

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Background. The prognosis of advanced pancreatic cancer is extremely poor and therefore a novel treatment strategy is desired. The authors thus started a prospective study of allogeneic reduced-intensity hematopoietic stem cell transplantation (RIST) for patients with advanced pancreatic cancer to evaluate the feasibility and efficacy of this approach for such patients.

Methods. Only patients with pathologically proven pancreatic cancer that was locally advanced or metastatic and not amenable to curative resection were included. The conditioning regimen consisted of gemcitabine, fludarabine, and busulfan.

Results. In the first stage of this study, the authors treated seven patients. Treatment-related mortality before day 100 was observed in one patient. The median survival after RIST was 229 days. An objective response on computed tomographic scan was observed in two patients and another had a tumor marker response. Marked tumor shrinkage was observed in one of the remaining patients after donor lymphocyte infusion. These antitumor effects appeared after the effect of the conditioning regimen had disappeared. In addition, some of these responses were associated with an increase in the serum anticarcinoembryonic antigen antibody level.

Conclusions. Pancreatic cancer appeared to be sensitive to a graft-versus-tumor effect; therefore, a larger clinical study with a refined strategy is warranted.

Keywords: Reduced-intensity stem cell transplantation, Minitransplantation, Pancreatic cancer, Graft-versus-tumor effect, Graft-versus-host disease.

(*Transplantation* 2005;79: 821–827)

Pancreatic cancer is the fifth most common cause of cancer-related mortality in Japan and the United States. The median duration of survival in advanced pancreatic cancer is less than 6 months, even when treated with gemcitabine (*1*), and therefore a novel treatment strategy is desired. Allogeneic nonmyeloablative or reduced-intensity hematopoietic stem cell transplantation (RIST) is a recently developed treatment approach for obtaining a graft-versus-tumor (GVT) effect without the toxicity associated with a myeloablative conditioning regimen (*2–6*). This treatment strategy is suitable for patients with solid tumors, because patients with advanced solid tumors are generally clinically infirm, and also a strong antitumor effect cannot be expected with an intensification of chemotherapy. In addition, a GVT effect has been noted in several solid tumors after conventional hematopoietic stem-cell transplantation (*7, 8*). Based on this background, RIST has been investigated for use against solid cancers since the late 1990s and its feasibility has already been demonstrated in several studies (*9–14*). However, there is still little information available regarding its efficacy against individual solid cancers. Childs et al. showed an excellent response rate of 53% after RIST against metastatic renal cell cancer (*9*). A GVT effect against renal cell cancer was confirmed in trials by other centers. In contrast, RIST against metastatic melanoma, which has been considered to be a good candidate for immunotherapy, resulted in a miserable outcome (*15*). Therefore, it is difficult to predict whether a GVT effect can be achieved against an individual tumor. We started a prospective study to evaluate the feasibility and efficacy of RIST against advanced pancreatic cancer after ethical approval in April 2002.

PATIENTS AND METHODS

Patients

Eligible patients were younger than 70 years of age and had pathologically proven pancreatic adenocarcinoma, which was locally advanced or metastatic and not amenable to curative resection. Patients had a human leukocyte antigen (HLA)-matched sibling or a family donor with a single mismatched HLA antigen. Patients with a poor performance status (Eastern Cooperative Oncology Group 2–4) and those with severely impaired organ function were excluded. Patients and donors gave their written informed consent to participate in this study.

Twelve patients with pancreatic cancer fulfilled the inclusion criteria but lacked a suitable donor. They were considered control patients. Four of them had metastatic lesions, whereas eight had locally advanced disease. Nine received

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chemotherapy with gemcitabine, whereas three were observed without chemotherapy.

Transplantation Procedure

Donors received granulocyte colony-stimulating factor at 200 $\mu g/m^2$ administered subcutaneously twice daily starting 3 days before the first collection of peripheral blood stem cells until the end of collection. Leukapheresis was performed daily until more than 2.0×10^6 $CD34^+$ cells/kg of the recipient's body weight were collected. Collected cells were then cryopreserved using standard techniques without ex vivo manipulation.

The conditioning regimen consisted of gemcitabine (1,000 mg/m^2/day as a 90-min infusion on days −16, −9, and −2) (*16*), fludarabine (30 mg/m^2/day as a 30-min infusion on days −8 to −3), and busulfan (4 mg/kg/day administered orally in four divided doses on days −6 and −5) (*4*, *17*). Graft-versus-host disease (GVHD) prophylaxis was performed with cyclosporine (CsA) (3 mg/kg/day as a continuous infusion) and short-term methotrexate (10 mg/m^2 on day 1 and 7 mg/m^2 on days 3 and 6). Frozen peripheral blood stem cells were thawed and infused on day 0. CsA was decreased at 10% per week from day 30 and discontinued by day 100 unless the patient developed acute GVHD. In patients with progressive disease without any evidence of acute GVHD, CsA was rapidly tapered over a 4-week period. Acute GVHD was graded as previously described (*18*). Grade II to IV acute GVHD was treated with methylprednisolone at 1 mg/kg per day, except for grade II GVHD limited to the skin, which was treated with topical steroid ointment.

Prophylaxis against bacterial, fungal, and *Pneumocystis carinii* infection consisted of tosufloxacin, fluconazole, and sulfamethoxazole-trimethoprim. As prophylaxis against herpes simplex virus infection, acyclovir was administered at 500 mg per day intravenously or 1,000 mg per day orally from days −7 to 35, followed by long-term low-dose (200 mg/day) oral administration (*19*). Patients received granulocyte colony-stimulating factor (filgrastim) at 300 μg per day by 3-hr infusion beginning on day 7 until the neutrophil count recovered to $500/mm^3$. Cytomegalovirus antigenemia assay using C10/C11 antibody was performed at least once per week after engraftment. Ganciclovir was started when more than two positive cells were detected on two slides (*20*, *21*).

Donor lymphocyte infusion (DLI) was performed for patients who had progressive disease and did not develop GVHD even after CsA was discontinued. The initial $CD3^+$ cell dose was 1 to 3×10^7 cells/kg and the dose was escalated every 4 weeks when the patient did not develop tumor response or GVHD.

Chimerism and Immunologic Analyses

Host-donor cell chimerism after transplantation was analyzed monthly by sex-chromosome fluorescent in situ hybridization or the short tandem repeat method after transplantation (*22*). The serum anti-carcinoembryonic antigen (CEA) antibody level was determined by enzyme-linked immunosorbent assay as previously described (*23*). Briefly, 96-well microplates were coated overnight at 4°C with a 5-μg/mL CEA preparation. The plates were washed and blocked for 2 hr at room temperature with 200 μL/well of a 0.1% Tween20, 5% nonfat dry milk, phosphate-buffered saline solution to prevent nonspecific binding. After the plates were washed further, 50 μL of 1:100 diluted patient sera was added per well and incubated for 2 hr at room temperature. After the plates were washed five times, 50 μL of horseradish peroxidase-labeled anti-human immunoglobulin G antiserum at 0.1 μg/mL in blocking buffer was added per well. The plates were incubated for 90 min at room temperature and then washed five times. The conjugated anti-CEA antibody was detected by adding 100 μL of tetramethylbenzidine substrate per well, incubating for 30 min at room temperature, adding 50 μL of 2N H_2SO_4 per well to terminate the reaction, and measuring the absorbance at 450 nm. The net anti-CEA antibody absorbance was determined by subtracting the absorbance of a noncoated well from the gross absorbance.

Outcome Measures

The primary endpoint of this study was transplant-related mortality within 100 days after transplantation. The secondary endpoint was the tumor response within 6 months after transplantation. Toxicities associated with the conditioning regimen were graded according to the criteria of Bearman et al. (*24*). Objective tumor response was evaluated by an independent radiologist using a monthly computed tomographic (CT) scan. Complete response was defined as disappearance of all clinical evidence of tumor for a minimum of 4 weeks. Minor and partial responses were defined as decreases of 25% to 50% and greater than 50%, respectively, in the sum of the products of the maximum diameter and its perpendicular diameter of all measurable lesions for a minimum of 4 weeks (*1*). The tumor marker response was evaluated by biweekly measurement of the serum CA19-9 level, because imaging modalities including ultrasonography and CT scan are

TABLE 1. Characteristics of the patients

Patient	Age/sex	Prior treatment	Duration from Dx to transplant (mo)	Meta	HLA	No. of $CD34^+$ cells
1	48/M	Gem	3	—	6/6	4.8×10^6/kg
2	40/M	Gem+RT, Gem	9	—	6/6	5.1×10^6/kg
3	57/F	Gem+CDDP	4	Liver	6/6	4.0×10^6/kg
4	36/F	Gem	3	Liver	6/6	2.9×10^6/kg
5	59/M	Gem	2	—	6/6	5.6×10^6/kg
6	66/F	Gem	2	Peritonitis	6/6	2.0×10^6/kg
7	61/M	Gem+RT	12	Liver	6/6	3.0×10^6/kg

Dx, diagnosis; Meta, metastatic lesion; Gem, gemcitabine; RT, local radiation; CDDP, cisplatin.

TABLE 2. Outcome after RIST

Patient	Donor chimerism (%)	aGVHD	Objective response	Final outcome
1	100	III	MR	Died as a result of bacteremia on day 192
2	100	II	SD	Died as a result of PD on day 293
3	100	II[a]	MR[d]	Died as a result of PD on day 262
4	100	II[a]	PD	Died as a result of PD on day 72
5	100	II	SD[c,d]	Died as a result of PD on day 587
6	100	III[b]	PD	Died as a result of PD on day 229
7	100	III[a]	SD	Died as a result of pneumonia on day 83

[a] GVHD that occurred after the rapid tapering of immunosuppressants.
[b] GVHD that occurred after DLI.
[c] Partial response on tumor marker evaluation.
[d] Morphine was discontinued.
aGVHD, acute graft-versus-host disease; MR, minor response; SD, stable disease; PD, progressive disease; DLI, donor lymphocyte infusion.

not sufficient to determine the accurate tumor size of pancreatic cancer (*25*). For patients with a normal value of CA19-9 before RIST, CEA, Dupan-II, or Span-I was measured instead. A complete marker response was defined as normalization of the tumor marker for a minimum of 4 weeks. Minor and partial responses were defined as decreases of 25% to 50% and greater than 50%, respectively, in the tumor marker for a minimum of 4 weeks.

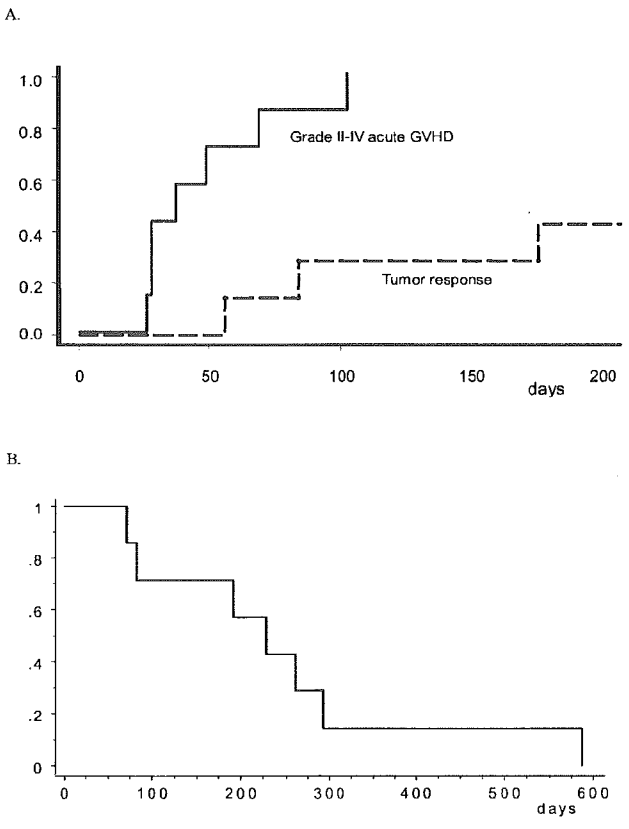

FIGURE 1. (A) Cumulative incidence of grade II to IV acute GVHD (*solid line*) and tumor response including both the objective response and the tumor marker response (*broken line*). (B) Overall survival after RIST against advanced pancreatic cancer.

Statistical Considerations

We defined success as the absence of early transplant-related mortality and planned seven and nine patients in the first and second stages of the study, with target and lower success rates of 80% and 50% and α and β errors of 10% and 10%, respectively (*26*). This is an analysis of the seven patients in the first stage. The cumulative incidence of tumor response was calculated by Gray's method considering death without response as a competing risk (*27*).

RESULTS

Patients

In the first stage of this study, seven patients with a median age of 57 years (range, 36–66 years) underwent RIST (Table 1). The duration from diagnosis to transplantation was 2 to 12 months. Four had metastatic disease and three had locally advanced disease. Prior treatment consisted of chemotherapy mainly with gemcitabine without an objective response, except for one patient (patient 2) who achieved a transient partial response after gemcitabine combined with local irradiation. All of the patients had progressive disease just before the conditioning regimen. All received a peripheral blood stem-cell graft from an HLA-identical sibling donor. The median number of $CD34^+$ cells in the graft was 4.0×10^6 cells/kg recipient body weight (range, 2.0–5.6 cells/kg). Three patients (patients 2, 5, and 6) underwent DLI for tumor progression 221, 336, and 69 days after transplantation, respectively. The dose of infused $CD3^+$ cells ranged between 2.7×10^7 and 1.8×10^8 cells/kg.

Regimen-Related Toxicity, Engraftment, and GVHD

Regimen-related toxicities were generally mild and well tolerated. Grade II to IV toxicity according to Bearman's grade was observed in two patients. One developed mild hepatic veno-occlusive disease, which recovered spontaneously. Another developed ileus caused by the pancreatic head tumor during the neutropenic period, which required nasogastric suction.

The median number of infused $CD34^+$ cells was 4.0×10^6 cells/kg (range, $2.0–5.6\times10^6$ cells/kg). Neutrophil engraftment was obtained within 12 days (range, 11–12 days)

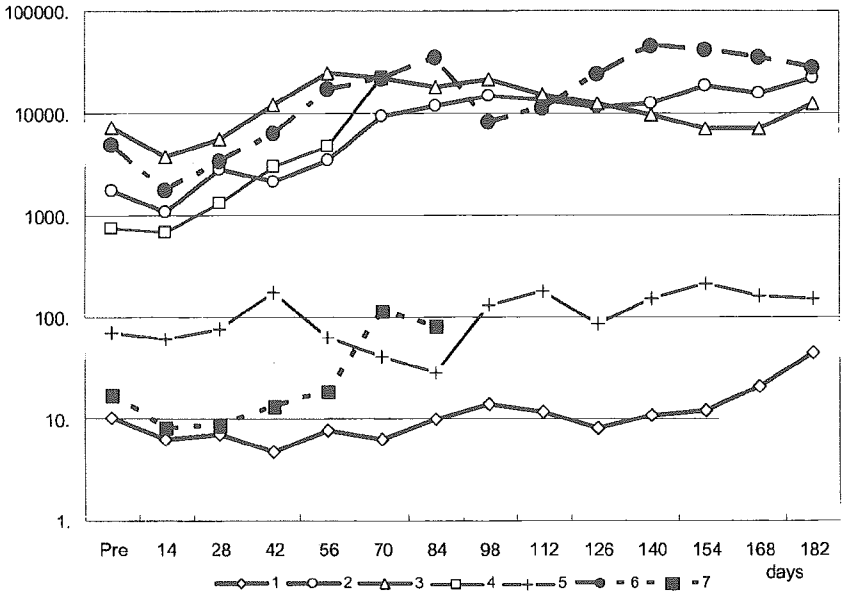

FIGURE 2. Serial changes in the values of tumor markers. CA19-9 was used all except patients 1 and 7, in whom CEA was measured instead because the CA19-9 level was within normal limits before transplantation. The units for the y-axis are units per milliliter for CA19-9 and nanograms per milliliter for CEA. A log scale was used for the y-axis to show serial data of all patients in one figure. Pre, pretransplant level.

after transplantation. Complete donor chimerism (>95%) was achieved in all patients by day 28 and maintained thereafter (Table 2). Grade II to III acute GVHD was observed in three patients (patients 1, 2, and 5) without rapid tapering of CsA, in three (patients 3, 4, and 7) after rapid tapering of CsA, and in one after DLI (Fig. 1A). Acute GVHD limited to the skin was cured with topical steroid only, whereas gut GVHD was successfully treated with systemic steroid.

Transplant-Related Mortality and Survival

Transplant-related mortality within 100 days after transplantation was observed in 1 patient (patient 7), who died as a result of pneumonia on day 83. Another patient (patient 1) died with bacteremia on day 192 caused by bacterial cholangitis. The other five patients died as a result of progressive disease. Median survival after RIST was 229 days (Fig. 1B), which was longer than the median survival of control patients after they were referred to our hospital (125 days), but this difference was not statistically significant.

Tumor Response

An objective minor response on CT scan was seen in two patients (patients 1 and 3) (Table 2 and Fig. 1A). Another patient (patient 5) achieved a partial tumor marker response. Two (patients 3 and 5) of the responders became free from all analgesics after achieving tumor regression.

As shown in Figure 2, the serum CA19-9 or CEA level generally increased within 6 to 8 weeks after transplantation after a transient decline associated with the conditioning chemotherapy. However, it stabilized (n=1) or began to decrease (n=3) thereafter, associated with the discontinuation of CsA or the development of GVHD. This suggests that the antitumor effect was caused by a GVT effect, not a chemotherapy effect. In particular, the serum CA19-9 level decreased from 25,180 U/mL on day 56 to 7,100 U/mL on day 154, with a tumor shrinkage on CT scan in patient 3 after the development of gut GVHD on day 69 (Fig. 3A). Evidence of a GVT effect against pancreatic cancer was also clearly seen in patient 6, who underwent DLI on day 69 for a progressive peritoneal metastatic lesion. The serum CA19-9 level decreased from 35,160 U/mL to 8,140 U/mL in 1 month, with an improvement of the peritoneal lesion on abdominal CT scan (Fig. 3B). However, these tumor responses were not durable and the response duration was between 28 and 98 days.

Serum Anti-CEA Antibody Level

Serum anti-CEA antibody levels were sequentially measured before and after RIST. The serum anti-CEA antibody level before RIST was higher than that in a normal population (n=3), with borderline significance (optical density, 0.109 ± 0.065 vs. 0.028 ± 0.030; $P=0.076$). It generally decreased 1 month after RIST with conditioning chemotherapy. Thereafter, an increase in the serum anti-CEA antibody level was observed in three patients. All three of these patients showed a tumor response, including one after DLI, whereas only one of the four patients without an increase in the serum anti-CEA antibody level showed a response. As shown in Figure 4, the increase in the serum anti-CEA antibody level was simultaneously associated with, or followed by, a decrease in the tumor marker level. However, this response was suppressed by the administration of high-dose steroid (Fig. 4A).

The increase in the serum anti-CEA level did not reflect nonspecific immune recovery, because we did not observe a significant relationship between serum anti-CEA antibody levels and antibody levels against other viral antigens (data not shown). In addition, the increase in the serum anti-CEA level did not result from a decrease in the serum CEA antigen that may absorb anti-CEA antibody, because we did not observe a negative relationship between them (data not shown).

DISCUSSION

In this study, we showed that RIST is a feasible treatment for patients with advanced pancreatic cancer. In addition, an objective response and a tumor marker response were observed in two and one of seven patients, respectively, who

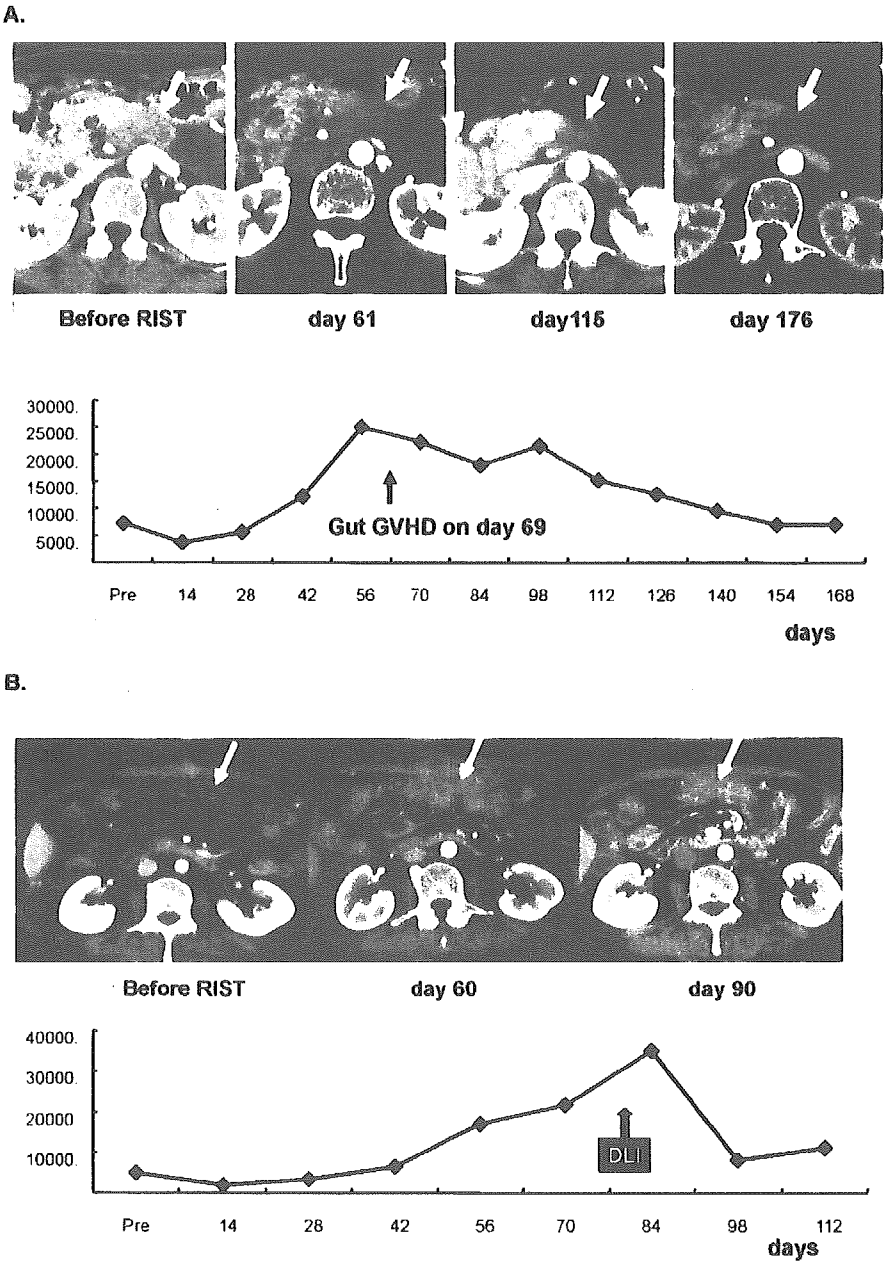

FIGURE 3. (A) Serial abdominal CT scans and serum CA19-9 levels of a patient (patient 3) who achieved an objective response after transplantation. The pancreatic body tumor shrunk and the serum CA19-9 level decreased after the development of gut GVHD. (B) Serial abdominal CT scans and serum CA19-9 levels of a patient (patient 6) who achieved an objective response after DLI. The peritoneal lesion worsened in the first 2 months after transplantation. However, the tumor began to shrink and the serum CA19-9 level rapidly decreased after DLI.

had not responded to conventional treatments. A response to DLI was seen in another patient. Although it is too early to evaluate survival after RIST, the median survival of 229 days might be better than that after conventional treatments. However, the tumor response was not durable, and all of the responding patients eventually died as a result of tumor progression.

It has been shown that at least 2 months are required to obtain a GVT effect after RIST against solid cancers. Childs et al. reported that tumor growth was frequently observed during the first few months after RIST for renal cell cancer (*9*). Therefore, we added gemcitabine to the combination of fludarabine and busulfan, a widely used conditioning regimen in RIST (*4*, *17*), to suppress tumor progression before the emergence of a GVT effect, because pancreatic cancer progresses much faster than renal cell cancer. A synergistic antitumor effect with the combination of gemcitabine and fludarabine has been demonstrated in in vitro studies (*28*).

It can be difficult to distinguish between a chemotherapy effect and a GVT effect after RIST. In this study, however, a transient chemotherapy effect attributable to the conditioning regimen was observed as tumor marker regression during the first 2 weeks after RIST. Thereafter, tumor marker began to increase, which suggested that the chemotherapy effect did not persist for longer than 2 weeks. The second regression of tumor marker was observed at least 2 months after RIST, associated with the discontinuation of CsA, the development of GVHD, or DLI. An objective tumor response was also observed at least 2 months after RIST. Therefore, the GVT effect

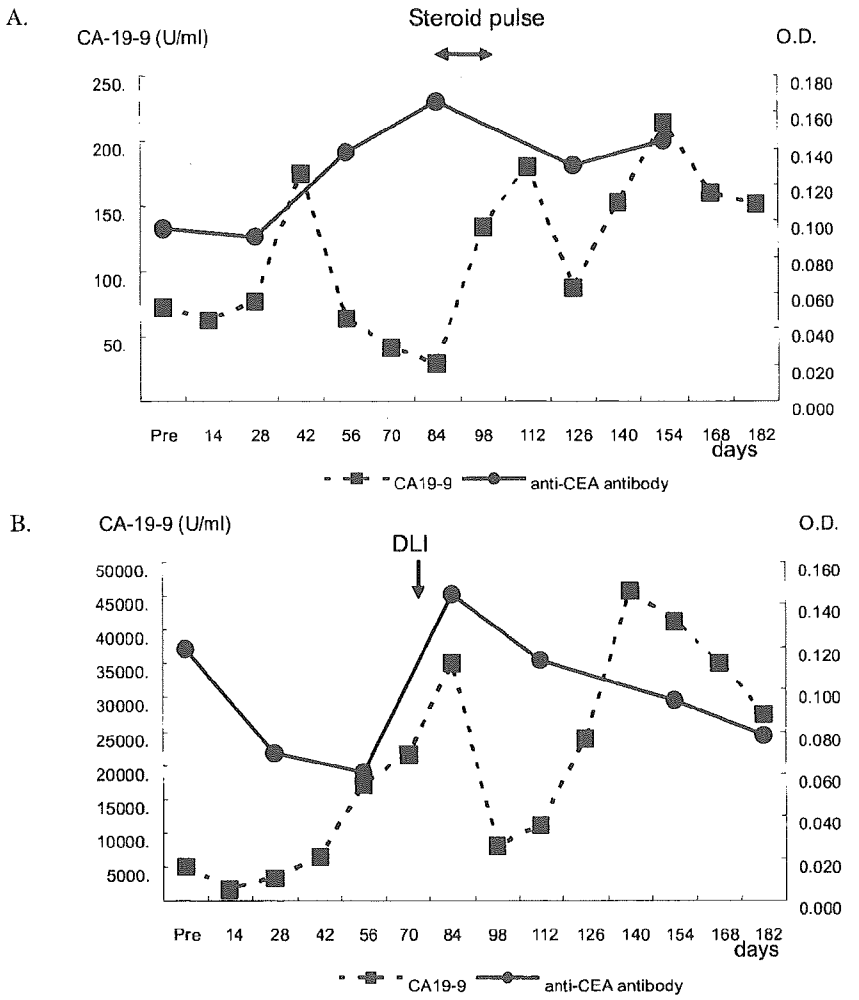

FIGURE 4. Serial changes in the serum CA19-9 level and anti-CEA antibody level. (A) Patient 5, who achieved a partial response on tumor marker evaluation. (B) Patient 6, who showed a tumor response after DLI.

against pancreatic cancer was clearly distinguished from the chemotherapy effect in this study.

Although a GVT effect against pancreatic cancer was seen in this study, this effect did not persist for more than 3 months. In addition, the development of GVHD was required to achieve a GVT effect. Therefore, we need a strategy for maintaining the GVT effect for a longer period without aggravating GVHD. To accomplish this, a specific immunotherapy against pancreatic cancer is required. Candidate target antigens include CA19-9, CA242, CEA, Her-2, mutated K-ras, and MUC-1 (*29*). Among these, CEA is attractive, because it is expressed in 85% to 90% of pancreatic cancer (*29*), and a specific immunotherapy against CEA could also be applied to other gastrointestinal cancers. Therefore, we retrospectively measured serial serum anti-CEA antibody levels in these patients. An increase in the serum anti-CEA antibody level after a transient decline just after RIST was associated with a tumor response. An elevation of serum anti-CEA antibody levels has already been demonstrated after a vaccine therapy targeting CEA (*23, 30*). Also, the presence of anti-CEA antibody at diagnosis was associated with better survival in patients with colon cancer, and thus anti-CEA antibody was suggested to have biologic significance (*31*). Thus, the GVT effect against pancreatic cancer in this study might be in part attributable to the specific immunity against CEA antigen. We also tried to detect CEA-specific cytotoxic T cells by enzyme-linked immunospot assay, but failed, probably because of the use of frozen peripheral blood mononuclear cells.

A major difference between RIST for hematologic malignancies and that for pancreatic cancer is the complications after transplantation. One patient (patient 1) experienced repeated episodes of biliary stent obstruction and bacterial cholangitis. Another two patients (patients 2 and 7) developed intestinal obstruction, probably because of the pancreatic head tumor. Therefore, a clinical trial of RIST for pancreatic cancer should be performed in close cooperation with the transplantation staff and gastroenterologists.

CONCLUSION

RIST appeared to be a feasible treatment for patients with advanced pancreatic cancer. The existence of a GVT effect against pancreatic cancer was strongly suggested by this study, but this effect was not durable and required the development of GVHD. We need to refine the strategy, for example, by the combination of specific immunotherapy against CEA after RIST.

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