SUPPLEMENTAL MATERIAL AND DATA

Mesothelin chimeric antigen receptor (CAR) T cells stimulate anti-tumor activity against metastatic pancreatic carcinoma

Gregory L. Beatty [1,3,*], Mark H. O'Hara[1,3], Simon F. Lacey [1,4,6], Drew A. Torigian [1,5], Farzana Nazimuddin [1,4,6], Fang Chen [1,4,6], Irina M. Kulikovskaya [1,4,6], Michael C. Soulen [1,5], Maureen McGarvey [1], Anne Marie Nelson[1], Whitney L. Gladney [1], Bruce L. Levine [1,4,6], J. Joseph Melenhorst [1,4,6], Gabriela Plesa [1], and Carl H. June [1,4,6,7]

[1]Abramson Cancer Center; University of Pennsylvania, Philadelphia, PA

[2]Division of Pulmonary, Allergy, and Critical Care, Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

[3]Division of Hematology-Oncology, Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

[4]Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

[5]Department of Radiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

[6]Center for Cellular Immunotherapies, University of Pennsylvania, Philadelphia, PA

[7]Abramson Family Cancer Research Institute, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

SUPPLEMENTAL RESULTS AND DISCUSSION

Safety of CARTmeso cellular therapy

We evaluated adverse events experienced from CARTmeso cellular therapy according to CTCAE v4.0. The most common adverse events (AEs) (all grades) reported included fatigue (67%), dysgeusia (50%), and abdominal pain (50%) (Supplementary Table 2). Dysgeusia was most likely related to known effects of DMSO that was used to cryopreserve the CARTmeso cell product. One patient (08212-113), who presented at baseline prior to CARTmeso cell infusion with a pancreatic head mass and multiple metastases involving the mesentery, liver and lung, experienced a cerebral vascular accident associated with disseminated intravascular coagulation and deep venous thrombosis after tolerating 8 of the 9 planned CARTmeso cell infusions. This patient's treatment course was complicated by worsening fatigue, anorexia, and pain. In addition, there was a notable rise in CA19-9 from baseline of 144,787 to 622,750 at the completion of the CARTmeso cell infusions. FDG-PET/CT imaging performed on day 23 after beginning CARTmeso cell therapy showed a complete metabolic response in liver metastases despite continued FDG uptake detected in the primary pancreatic lesion. At that time, the patient also presented with a new expressive aphasia and a follow-up MRI of the head performed on day 24 revealed numerous acute infarcts throughout the brain bilaterally with ultrasound dopplers revealing evidence of acute deep vein thrombosis bilaterally in the lower extremities. Given the temporal relationship of this event to the investigational treatment, these AEs were considered "possibly related" but were more likely to be a result of disease progression that was seen on imaging and consistent with a rising CA19-9 and worsening disease-related symptoms. The final infusion of CARTmeso cells was not administered to this patient due to hospitalization and medical management of AEs.

Clinical activity of CARTmeso cells

For patient 08212-113, we observed a complete metabolic reduction in FDG uptake by PET/CT imaging in all liver lesions at Month 1 compared to baseline (Figure 1E, F). However, the metabolically active volume seen within the pancreas at Month 1 was found to increase (206% increase), despite a slight decrease in SUV_{max} of the primary pancreatic lesion (6.59 to 3.09). A similar swelling of lesions was also seen in the liver at Month 1 on unenhanced CT imaging (Figure 1E). Each of these lesions showed a loss of FDG uptake. This mixed tumor response implies distinct biology associated with the primary and metastatic lesions that may regulate the efficacy of CARTmeso cells *in vivo*. Analysis of a baseline metastatic lesion from this patient showed cell surface expression of mesothelin on malignant cells (Figure 2D) and the presence of tumor-infiltrating T cells, suggesting that CARTmeso cells may have been capable of penetrating the microenvironment of metastatic lesions in this patient. Tissue from the primary pancreatic lesion, though, was not available for analysis. As a result, we can only speculate that the mixed metabolic response seen in this patient may reflect (i) differences in target antigen expression between metastatic and primary tumors; (ii) the reduced capacity of CAR T cells to effectively infiltrate primary lesions; or (iii) mechanisms of immune suppression which may differ between primary and metastatic lesions. Nonetheless, these findings highlight the potential role of tumor heterogeneity in regulating the efficacy of T cell immunotherapy in PDAC.

CARTmeso cell persistence *in vivo*

CAR expression within the peripheral blood was detected transiently after each infusion (Figure 2A). We have previously reported the emergence of human anti-mouse antibodies detected after infusion of CARTmeso cells in patients [1]. Although we did not detect the emergence of

human anti-mouse antibodies (HAMA) in any of the patients after CARTmeso infusion, human anti-chimeric antibodies (HACA) were seen in some patients. Specifically, we detected increased levels of HACA in 08212-113 (IgG, IgA, and IgM), 08212-111 (IgG), and 08212-104 (IgG, and IgA) (Supplementary Figure 2A). HACA levels were not detectable at multiple defined time points after CARTmeso infusion in patients 08212-100, 08212-108 or 08212-114. The lack of HAMA and HACA responses in some patients indicates that humoral responses against the CAR cannot explain the transient persistence of CARTmeso cells seen across all patients. While transient persistence of CARTmeso cells in the peripheral blood could reflect trafficking to tissues or lymphoid organs, it is likely that this finding, at least in part, is due to cell division and loss of expression of the mRNA CAR which is expected to be short-lived based on *in vitro* studies [2]. However, since the completion of this clinical study, we have now conducted a second trial evaluating the adoptive transfer of CARTmeso cells engineered to stably express a mesothelin-specific CAR using lentiviral technology [3]. In this study, we also observed transient persistence of lenti-CARTmeso cells in the peripheral blood. We are currently investigating whether this poor persistence reflects immune-mediated rejection, trafficking, or poor functional capacity of T cells isolated from PDAC patients as has been reported in chronic lymphocytic leukemia (CLL) [4].

CARTmeso cell infusion is associated with humoral immune responses

We evaluated the impact of CARTmeso cell infusion on endogenous humoral immune responses using protoarray technology. Changes in reactive IgG antibody levels (≥2 fold increase) between baseline and Month 1-2 were evaluated for all patients. The maximum response was seen in patient 08212-113 who demonstrated an increase in IgG antibodies reactive against 115 proteins. Across all patients except patient 08212-108, reactive IgG antibodies were detected that increased more

than 2-fold above baseline levels (Figure 2C). For patient 08212-108, there was no change in self-reactive IgG antibodies detected at 2 months or 4 months after beginning CARTmeso therapy. We also detected increases in IgG antibodies reactive against three immune-related proteins (BCMA, PD-1, and PD-L1) in multiple patients (Supplementary Figure 2D). While the mechanism and implications of this immune response elicited by CARTmeso cells is uncertain, it could reflect the capacity of activated T cells to stimulate B cells, possibly via CD40L on activated CARTmeso cells which may engage CD40 on B cells. It is also possible that these changes reflect tumor progression independent of therapy. However, the development of self-reactive antibodies in the setting of vaccination has been previously seen in patients with PDAC treated with a granulocyte-macrophage colony-stimulating factor-secreting allogeneic PDAC vaccine. In this case, neutralizing antibodies to galectin-3, a negative regulator of antigen-specific T cell activation, were detected after immunization in patients responding to treatment [5].

CARTmeso cells stimulate systemic cytokine release

For patient 08212-113, CARTmeso cell infusion was associated with a rapid release of systemic cytokines initially detected within 1 hour of infusion. We found that the levels of several inflammatory cytokines, including IL-6, hepatocyte growth factor (HGF), interleukin-1 receptor antagonist (IL-1RA), and IL-8, increased significantly after starting therapy in patient 08212-113, but not in any of the other patients (Figure 2A). In addition, no changes in levels of cytokines commonly associated with T cell activation, including IL-2, IL-4, IL-5, IL-7, IL-17, IL-21, tumor necrosis factor (TNF), and interferon gamma (IFN-γ) were noted among an array of 30 soluble factors (see Supplementary Methods).

We previously reported systemic cytokine release induced with repeated CARTmeso cell infusion in a patient with metastatic PDAC (21211-101) [6]. Similar to patient 08212-113, CARTmeso cell therapy produced some anti-tumor activity in patient 21211-101 as well as a spreading of antibody responses against multiple protein targets [6]. However, the cytokine response detected in the serum of 21211-101 was distinct from 08212-113. Whereas we detected significant changes in IL-12, RANTES, MCP-1 and MIP-1beta for patient 21211-101, we detected no significant changes in these cytokines for patient 08212-113. In contrast, in the current study, we detected increases in IL-6 and IL-8 for patient 08212-113, whereas systemic release of these cytokines was not seen in patient 21211-101. We can only speculate that the differences in the systemic cytokine release detected may reflect distinct myeloid phenotypes stimulated by therapy in the two patients, as each of the observed cytokines is consistent with an innate immune response. For example, the lack of production of IL-12 but increase in IL-8 seen in patient 08212-113 may reflect activation and mobilization of a distinct phenotype of myeloid cells.

SUPPLEMENTAL METHODS

Patients

Between August 2013 and October 2014, ten patients with chemotherapy-refractory metastatic pancreatic ductal adenocarcinoma were enrolled at the Abramson Cancer Center, University of Pennsylvania (Philadelphia, PA) (Table 1 and Supplementary Figure 1). Two patients withdrew from the study due to disease progression prior to receiving treatment. One patient missed apheresis which was necessary for CARTmeso cell manufacturing. Sufficient CARTmeso cell product could not be produced for one patient due to poor T cell expansion *in vitro*. Overall, six patients with measurable metastatic disease were treated on study with autologous T cells

electroporated with a chimeric antigen receptor recognizing mesothelin. Inclusion criteria were histologically confirmed metastatic pancreatic adenocarcinoma, age $\geq$ 18 years, Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1, life expectancy > 3 months, evidence of metastatic disease, failure of at least 1 prior chemotherapy for metastatic disease, measurable disease as defined by RECIST 1.1 criteria, satisfactory organ and bone marrow function, and normal blood coagulation parameters. Patients were also required to have adequate venous access for subsequent CAR T cell administration. Exclusion criteria included receipt of an investigational study drug within 28 days (42 days for non-murine monoclonal antibodies), active invasive cancer other than pancreatic adenocarcinoma (except active non-invasive cancers), active autoimmune disease requiring immunosuppressive therapy within the past 4 weeks, ongoing or active infection, concurrent treatment with systemic high dose corticosteroids, prior gene therapy or therapy with murine monoclonal antibodies or products of murine origin, concurrent treatment with any anticancer agent, history of allergy to murine proteins, and history of allergy or hypersensitivity to study product excipients (i.e. human serum albumin, DMSO, and Dextran 40). Patients with any clinically significant pericardial effusion; CHF (NY Heart Association Grade II-IV), or cardiovascular condition that would preclude assessment of mesothelin-induced pericarditis were excluded. Subjects on active anti-coagulation therapy and pregnant women were also excluded. All patients provided written informed consent and the study was approved by the institutional review board of the University of Pennsylvania.

Study Design and Treatment Plan

This was a Phase I safety and feasibility study (NCT01897415). The primary objective was to determine the safety and manufacturing feasibility of autologous chimeric antigen receptor (CAR) T cells transfected with anti-mesothelin messenger RNA (mRNA) expressing a single chain antibody variable fragment linked to the intracellular CD3ζ T cell receptor domain and the 4-1BB co-stimulatory domain. Secondary objectives were to assess clinical anti-tumor activity and to evaluate (i) short-term peripheral persistence and trafficking of CAR T cells, (ii) cytokine responses, (iii) development of general humoral and cellular anti-tumor responses, and (iv) development of host anti-CAR immune responses.

Subjects were initially screened for eligibility before undergoing large-volume leukopheresis to obtain peripheral blood mononuclear cells (PBMC) for CAR T cell manufacturing. To model CAR T cell persistence *in vivo*, subjects received intravenous administration of 1 to 3 x $10^8/m^2$ T cells three times weekly (M-W-F) for three weeks. Lymphodepletion was not incorporated in this study as the priority was to establish safety before considering strategies to optimize efficacy. Tumor response was assessed by computed tomography (CT) performed at baseline and 2 months according to Response Evaluation Criteria in Solid Tumors (RECIST) 1.1. Tumor assessments also included ^{18}F-2-fluoro-2-deoxy-D-glucose positron emission tomography/computed tomography (FDG-PET/CT) imaging performed at baseline and at the end of therapy. Optional tumor biopsies of accessible lesions were performed at baseline and within 3-7 days after the last planned dose of RNA CAR T cells. Peripheral blood samples were obtained at defined time points to monitor for measures of safety and efficacy. All authors had access to the study data and reviewed and approved the final manuscript.

Safety Assessments

All patients who received CARTmeso cells were evaluated for safety. Safety assessments included the incidence of treatment-related adverse events (AEs) according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0. A dose-limiting toxicity (DLT) was defined as (i) any grade 3 or higher non-hematologic toxicity except asymptomatic Grade 3 electrolytes, Grade 3 nausea, vomiting, diarrhea or fatigue; Grade 3 non-hematologic toxicities that can be controlled to Grade 1 or less with appropriate treatment were not considered dose limiting; (ii) Grade 2 or higher autoimmune toxicity, including pericarditis, peritonitis, or pleuritis; and (iii) Grade 3 or higher allergic reaction, or grade 2 allergic reactions in which symptoms reappear after repeat infusion. AE reporting began on Day 0 and continued through approximately 3 months after the last infusion. Subjects were continually reassessed for evidence of acute and cumulative toxicity. Dysgeusia reported as an AE was most likely related to known effects of DMSO that was used to cryopreserve the CARTmeso cell product.

Sample collection and processing

Research sample processing, freezing, and laboratory analyses were performed in the Translational and Correlative Studies Laboratory (TCSL) at the University of Pennsylvania. TCSL operates under principle of Good Laboratory Practice based on standard operating procedures (SOP) and protocols for sample receipt, processing, storage, and analysis. The described assays and data reports conform to MIATA guidelines [7]. Peripheral blood samples were collected in vacutainer tubes (Becton Dickinson): lavender top (K2EDTA) tubes to obtain cells and red top (no addition) tubes to obtain serum. Research tubes were delivered to TCSL within 2 hours of blood collection for processing according to established SOPs. Peripheral blood mononuclear cells were purified,

processed and stored at -140°C, whereas serum was isolated and stored at -80ºC. RNA was isolated directly from whole blood using Ribopure blood kits (Ambion).

CARTmeso cell manufacturing

T cells were manufactured according to a U.S. Food and Drug Administration (FDA)-accepted IND. T cells were isolated by elutriation from a large-volume apheresis. Cells were expanded *in vitro* in cell culture medium supplemented with human serum for 10 $\pm$ 2 days using bead-immobilized anti-CD3 and anti-CD28 antibodies. Expanded T cells were then electroporated with anti-mesothelin ss1 scFv CAR mRNA construct containing the 4-1BB and CD3ζ signaling domains, using a closed system electroporation device (Maxcyte Inc) as described previously [6]. The CAR transfected autologous T cell product was then cryopreserved in an infusible cryoprotectant-supplemented solution. The infused cells were an average of 95.7% $CD3^+$ and 93.7% $scFv^+$ as assessed by flow cytometry. The % of infused cells expressing CD4, CD8 and scFv as well as the CD4:CD8 ratio of the infused cell product for each patient was as follows:

Patient	%CD3+ CD45+	%CD3+ CD4+	%CD3+ CD8+	CD4:CD8 ratio	%scFv
08212-100	92.5%	65.7 %	36.0%	1.8	98.6%
08212-104	93.1%	67.2%	25.0%	2.7	92.0%
08212-108	98.7%	87.5%	14.6%	6.0	97.8%
08212-111	95.6%	70.7%	30.7%	2.3	93.8%
08212-113	97.8%	78.6%	24.8%	3.2	87.2%
08212-114	96.4%	68.4%	32.0%	2.1	93.1%

The cell viability was 90.7% (mean, with a range of 76.9% to 95.9% among all products). One manufacturing failure was encountered (Supplemental Figure 1B) due to limited *in vitro* expansion of T cells and poor cell viability (41% viable cells) after *in vitro* electroporation and cryopreservation. As a result, the CAR T cell product for this patient did not meet release criteria for infusion, specifically a minimum of 70% cell viability and sufficient cells to complete 9

infusions was a protocol requirement. Frozen CARTmeso cells were thawed at the time of cell infusion and administered to patients in the Clinical Trials Research Center at the Hospital of the University of Pennsylvania.

PET Data Acquisition and Analysis

FDG-PET/CT was performed on all patients at baseline and at Month 1 using a multidetector row whole-body PET/CT scanner with time-of-flight capabilities (Gemini TF, Philips Medical Systems, Bothell, WA). Patients had fasted for > 6 hours and plasma glucose levels were < 200 mg/dl. At approximately 60 minutes prior to imaging, ~15 mCi of FDG were administered intravenously. 3D PET images were acquired from the skull vertex to the toes. Rescaled low-dose CT images were used for attenuation correction of the PET images. Images were analyzed using an automatic adaptive thresholding method (ROVER, ABX GmbH, Radeberg, Germany) with an initial threshold setting of 40% of maximum lesional metabolic activity and other settings on default to delineate FDG avid lesions. Metabolically active volumes for individual lesions from PET datasets were determined and summed to calculate the total metabolically active volume as previously described [8, 9].

Real-time quantitative PCR analysis of RNA transcripts

iScript™ Reverse Transcription Supermix for RT-qPCR (Biorad) was used to synthesize cDNA from whole blood RNA. The abundance of transgene and CD3ε transcripts was determined using quantitative PCR (qPCR) assays from 50 ng of cDNA per replicate with three replicates for each data point. Primer/probes that detect the 4-1BB-CD3ζ junctional fragment were used to quantify transgene abundance as previously described [6]. CD3ε transcripts were quantified using TaqMan

assay (Life Technologies). Quantification of levels of T cells and transgene samples was determined in relation to a 9-point standard curve derived from the infusion product of each patient, as previously described [6].

Analysis of serum soluble factors

At baseline, day 0 and defined time points after CARTmeso adoptive cell therapy, whole blood was collected in red top tubes without additive, processed to obtain isolate serum, and stored at -80°C in single use aliquots. Luminex bead array technology and 30-plex kits (Cat# LHC6003M, Life Technologies, Carlsbad, CA) were used according to manufacturer instructions to quantify the following soluble serum factors: (IL-1RA, FGF-Basic, MCP-1, G-CSF, IFN-γ, IL-12, IL-13, IL-7, GM-CSF, TNF-α, IL-1β, IL-2, IL-4, IL-5, IL-6, IFN-α, IL-15, IL-10, MIP-1α, IL-17, IL-8, EGF, HGF, VEGF, MIG, RANTES, Eotaxin, MIP-1β, IP-10, and IL-2R). Serum samples cryopreserved at -80°C from day -1 or baseline to day 28 were thawed and analyzed according to the manufacturers' protocols. Assay plates were measured using a FlexMAP 3D instrument (Luminex, Austin, TX), and data acquisition and analysis were done using xPONENT software (Luminex).

Data quality was examined based on the following criteria. The standard curve for each analyte has a R^2 value > 0.95 with or without minor fitting using xPONENT software. The results for the in-house control were required to be within the 95% of CI (confidence interval) derived from historical in-house control data for >25 of the tested analytes. No further tests were done on samples with results out of range low (<OOR). Samples with results that were out of range high (>OOR) or greater than two times the standard curve maximum value (SC max) were re-tested at higher dilutions. Results that passed the above quality controls or retests were used in this data set.

Detection of humoral immune responses

Humoral immune responses induced by CARTmeso treatment were evaluated using protoarray™ technology (Life Technologies). This high-throughput serological analysis was used to detect the presence of IgG antibodies reactive to an array of 9,480 unique protein probes. Serum samples at baseline and month 1 (patients 113 and 114) or month 2 (patients 100, 104, 108, and 111) were diluted 1:500 per manufacturer instructions. Gpr files containing signal intensities were analyzed using ProtoArray Prospector Software (v5.2, Life Technologies) to normalize intensities. Positive "hits" were defined based on a Z-factor > 0 and ratio > 2 comparing post-therapy samples against the patient-matched baseline pre-therapy sample. Where the signal intensity was less than the median MFI intensity for the plate (i.e. 198), values were adjusted to the median MFI. Gene ontology of differentially recognized proteins were classified according to their statistical significance (*p* value) using DAVID Functional Annotation Bioinformatics Microarray Analysis [10, 11]. Human anti-mouse antibodies (HAMA) were detected by ELISA and human anti-chimeric antibodies (HACA) were detected using a flow cytometry-based assay as previously described [1].

Immunohistochemistry

Tissues from tumor biopsies obtained at baseline were fixed in 10% formalin and paraffin embedded. Sections were stained with hematoxylin and eosin or with a CD3 or mesothelin-specific primary antibody (clone 5B2, ThermoFisher) and detected using goat anti-mouse secondary antibody. Staining was detected using DAB and slides were counterstained with hematoxylin. Brightfield images were acquired on a BX43 upright (Olympus) microscope.

References

1. Maus MV, Haas AR, Beatty GL, Albelda SM, Levine BL, Liu X, Zhao Y, Kalos M, June CH. T cells expressing chimeric antigen receptors can cause anaphylaxis in humans. Cancer Immunol Res 2013;1:26-31.
2. Zhao Y, Moon E, Carpenito C, Paulos CM, Liu X, Brennan AL, Chew A, Carroll RG, Scholler J, Levine BL, Albelda SM, June CH. Multiple injections of electroporated autologous T cells expressing a chimeric antigen receptor mediate regression of human disseminated tumor. Cancer Res 2010;70:9053-61.
3. Tanyi JL, Haas AR, Beatty GL, Morgan MA, Stashwick CJ, O'Hara MH, Porter DL, Maus MV, Levine BL, Lacey SF, Nelson AM, McGarvey M, Kerr NDS, Plesa G, June CH. Abstract CT105: Safety and feasibility of chimeric antigen receptor modified T cells directed against mesothelin (CART-meso) in patients with mesothelin expressing cancers. Cancer Research 2015;75:CT105-CT105.
4. Ramsay AG, Johnson AJ, Lee AM, Gorgun G, Le Dieu R, Blum W, Byrd JC, Gribben JG. Chronic lymphocytic leukemia T cells show impaired immunological synapse formation that can be reversed with an immunomodulating drug. J Clin Invest 2008;118:2427-37.
5. Gardner RA, Finney O, Annesley C, Brakke H, Summers C, Leger K, Bleakley M, Brown C, Mgebroff S, Kelly-Spratt KS, Hoglund V, Lindgren C, Oron AP, Li D, Riddell SR, Park JR, Jensen MC. Intent-to-treat leukemia remission by CD19 CAR T cells of defined formulation and dose in children and young adults. Blood 2015;129:3322-3331.
6. Beatty GL, Haas AR, Maus MV, Torigian DA, Soulen MC, Plesa G, Chew A, Zhao Y, Levine BL, Albelda SM, Kalos M, June CH. Mesothelin-specific chimeric antigen receptor

mRNA-engineered T cells induce antitumor activity in solid malignancies. Cancer Immunol Res 2014;2:112-120.

7. Britten CM, Janetzki S, Butterfield LH, Ferrari G, Gouttefangeas C, Huber C, Kalos M, Levitsky HI, Maecker HT, Melief CJ, O'Donnell-Tormey J, Odunsi K, Old LJ, Ottenhoff TH, Ottensmeier C, Pawelec G, Roederer M, Roep BO, Romero P, van der Burg SH, Walter S, Hoos A, Davis MM. T cell assays and MIATA: the essential minimum for maximum impact. Immunity 2012;37:1-2.

8. Torigian DA, Lopez RF, Alapati S, Bodapati G, Hofheinz F, van den Hoff J, Saboury B, Alavi A. Feasibility and performance of novel software to quantify metabolically active volumes and 3D partial volume corrected SUV and metabolic volumetric products of spinal bone marrow metastases on 18F-FDG-PET/CT. Hell J Nucl Med 2011;14:8-14.

9. Hofheinz F, Potzsch C, Oehme L, Beuthien-Baumann B, Steinbach J, Kotzerke J, van den Hoff J. Automatic volume delineation in oncological PET. Evaluation of a dedicated software tool and comparison with manual delineation in clinical data sets. Nuklearmedizin 2012;51:9-16.

10. Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009;4:44-57.

11. Huang da W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 2009;37:1-13.