Two doses of humanized anti-CD25 antibody in renal transplantation

A preliminary comparative study

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HuCD25mAb is a humanized anti-CD25 antibody which has the same amino acid sequence as daclizumab (Zenapax, Roche). HuCD25mAb is expressed in Chinese hamster ovary (CHO) cells while daclizumab is expressed in the NSO myeloma cell line. A comparative study was performed to evaluate the pharmacokinetics and pharmacodynamics between huCD25mAb and daclizumab in a two-dose regimen incorporating triple immunosuppressant treatment regimens (MMF, CsA and steroids). Fifteen patients were enrolled and randomized to receive intravenous infusion of either huCD25mAb (n = 10) or daclizumab (n = 5) at a dosage of 1 mg·kg⁻¹ on operation day 0 and post-operation day 14. Serum concentrations of huCD25mAb and daclizumab were measured by a validated competitive ELISA. Subgroups of CD3⁺, CD25⁺, CD4⁺ and CD8⁺ lymphocytes were monitored periodically by flow cytometry. The concentration-time curves of huCD25mAb and daclizumab were found to fit well to a one-compartment model. A significant decline of proportion (%) of CD3⁻CD25⁺ and CD3⁺CD25⁺ lymphocytes was observed 30 min after first infusion on day 0 (3.40 ± 1.83 to 0.03 ± 0.07, 3.35 ± 2.02 to 0.37 ± 0.49), and these levels remained low for at least 70 days (0.03 ± 0.05, 0.31 ± 0.47). All pharmacokinetic parameters of huCD25mAb seemed similar to those of daclizumab. The two-dose huCD25mAb regimen was as effective as daclizumab in rapidly achieving high therapeutic concentration in the treated patients, and a significant decrease of CD3⁻CD25⁺ and CD3⁺CD25⁺ lymphocytes was demonstrated. This suggests that two-dose regimen is feasible in maintaining host immunosuppression and may provide an effective and economical strategy for reducing incidence of acute graft rejection.

Introduction

Activated T lymphocytes play a pivotal role in acute rejection of solid organ transplants. Modern immunosuppressive drugs such as cyclosporine and tacrolimus affect the immune response against allogenic tissue by inhibition of several T-cell activation genes. These immunosuppressive agents reduce the incidence and severity of acute rejection successfully. Simultaneously, they are associated with considerable side effects and risks of opportunistic infections because they lack specificity for activated T lymphocytes. More specific immunosuppressive regimens need to be developed to assure longer-term efficacy and minimize agent-specific side effects.

Interleukin-2 (IL-2) is a potent T cell growth cytokine which, in T cell activation, acts in an autocrine fashion to promote the growth, proliferation and differentiation of the T cell recently stimulated by antigen. High-affinity interleukin 2 receptors (IL-2R) on human T lymphocytes are multimeric complexes containing at least three subunits: IL-2Rα (CD25, Tac), IL-2Rβ (CD122) and IL-2Rγ (CD132). Only IL-2Rα is specific for IL-2, whereas IL-2Rβ and IL-2Rγ are parts of other cytokine receptors such as the IL-15R. Therefore, anti-CD25 targeted therapy seems to be a promising way for specific immunosuppression. Two marketed monoclonal antibodies (mAbs), daclizumab and basiliximab, target CD25.

Daclizumab (Zenapax®, Roche, USA), a humanized anti-CD25 antibody to the alpha chain (α) of the IL-2R (IL-2Rα), has proven...
effective and safe in several studies conducted in renal transplantation\(^1-^5\) and liver transplantation recipients.\(^6-^9\) Zenapax\(^\circledR\) has been routinely used at a dose of 1 mg·kg\(^{-1}\) for intravenous injection, with the first dose given in the early postoperative period and a subsequent dose to be followed every two weeks for a total of five doses.\(^4\) Basiliximab (Simulect\(^\circledR\), Novartis, Switzerland) is a chimeric monoclonal antibody specific for the alpha transmembrane protein of the interleukin-2 (IL-2) receptor on T cells. The optimal dosing regimen for basiliximab consists of two doses of 20 mg administered intravenously within 2 h before transplantation and on day 4 post-transplantation.\(^10\)

HuCD25mAb, a humanized anti-CD25 antibody which has the same amino acid sequence as daclizumab, was developed by Shanghai Key Lab., of Cell Engineering and Shanghai National Engineering Research Center for Antibody Medicine. HuCD25mAb is expressed in Chinese hamster ovary (CHO) cells while daclizumab is expressed in the NSO myeloma cell line. Our previous preclinical study indicated that huCD25mAb could inhibit the binding and biological activity of IL-2 on activated T lymphocytes like daclizumab and basiliximab. The present study attempted to determine whether the serum concentration of huCD25mAb in renal recipients with a 2-dose regimen could blockade interleukin-2 receptor (IL-2R) and provide sufficient therapeutic level for preventing acute graft rejection, and to compare the pharmacokinetic homogeneity between huCD25mAb and daclizumab.

**Results**

**Demographics.** The demographic characteristics of the participants in this study are summarized as follows. There were 12 males and three females with an age range from 18 to 56 years. The mean age was 35.8 years, and the mean weight of males and females was 69 kg and 52.3 kg, respectively. The etiology of end-stage renal disease (ESRD) was as follows: chronic glomerulonephritis (CGN) (n = 1), proteinuria (n = 1) and unexplained serum creatinine abnormality (n = 13). There was no imbalance between the two treatment groups in demographic parameters such as primary cause of renal failure, panel reactive antibodies and HLA mismatch. All patients received a total of two doses of anti-CD25 antibody; thirteen of them were completely investigated during the 70-day follow-up period. One patient in the daclizumab group developed acute rejection; one in the huCD25mAb group had delayed graft function (DGF) and was withdrawn from the trial after less than three weeks.

**Validity of the method for determination.** The ELISA assay was utilized to determine the anti-CD25 antibody levels in the serum samples. The pass/fail criteria for the assay were determined by the performance of QC in each assay. The results of the blank serum samples fortified with 50, 5 and 0.5 mg·L\(^{-1}\) tested huCD25mAb showed that the recovery rates were 99% ± 5.8%, 97% ± 6.8% and 99% ± 7.8%, respectively (n = 5, parallel experiments). Maximal coefficients of variation (CV\%) of intra-assay and inter-assay were 9.1% and 8.8%, respectively. Within the concentration range of 0.5–50 mg·L\(^{-1}\), the concentration of the anti-CD25 antibody could be calculated by four-parameter logistic regression. It is important to calculate the concentration in samples by the parameters obtained from the standard curve of the same microplate. We defined levels of anti-CD25 antibody that were not quantified as below the limit of quantification (LOQ). Samples that returned values above the LOQ were diluted into the assay range with pooled normal human serum and tested repeatedly in the assay. The validity of the method demonstrated that the ELISA assay was reliable for the determination of serum anti-CD25 antibody levels. Specificity, sensitivity, accuracy and precision all met the requirements for PK and PD study.

**Pharmacokinetics of daclizumab.** Serum anti-CD25 antibody concentration-time profiles of huCD25mAb and daclizumab are shown in Figure 1. Following the first dose of huCD25mAb on day 0, the serum concentration peaked at 15.17 ± 3.09 mg·L\(^{-1}\). After the second dose, the highest C\(_{\text{max}}\) of 17.77 ± 5.74 mg·L\(^{-1}\) was achieved on day 14. Trough levels (\(X \pm SD\)) (mg·L\(^{-1}\)) were 2.21 ± 1.30, 0.96 ± 0.76 on day 14 and 70 respectively. Subsequently, the serum concentration of daclizumab peaked at 12.36 ± 3.70 mg·L\(^{-1}\) after the first dose and the highest C\(_{\text{max}}\) 18.08 ± 6.66 mg·L\(^{-1}\) was achieved on day 14 after the second dose. Trough levels (\(X \pm SD\)) (mg·L\(^{-1}\)) were 2.10 ± 2.26, 0.82 ± 0.66 on day 14 and 70 respectively. Two sample t-test was performed to assess the correlation of serum antibody concentration at each time point between huCD25mAb and daclizumab. There was no significant difference statistically. The mean serum concentrations of antibody (huCD25mAb and daclizumab) at partial time points and the calculated pharmacokinetic parameters were shown in Table 1. There was no statistical difference between the pharmacokinetic parameters of huCD25mAb and daclizumab. Both antibodies exhibited one-compartmental pharmacokinetic behavior in this study. The elimination half-life (\(T_{1/2}\)) of huCD25mAb and daclizumab were 320 h and 315 h, respectively. The mean values for the volume of distribution (V\(_{\text{d}}\)) after the second dose were 0.0854 L·Kg\(^{-1}\) (huCD25mAb group) and 0.0760 L·Kg\(^{-1}\) (daclizumab group), respectively. The mean Cl of huCD25mAb was 0.204 mL·h\(^{-1}\)·Kg\(^{-1}\) while that of daclizumab was 0.177 mL·h\(^{-1}\)·Kg\(^{-1}\). Finally, the areas under the curve after the second dose (AUC\(_2\)) of huCD25mAb and daclizumab in this study were 6105.06 ± 2961.78 mg·h·L\(^{-1}\) and 6212.63 ± 2387.46 mg·h·L\(^{-1}\).

**Lymphocyte subgroups proportion (%)**. The effect of anti-CD25 antibody on circulating lymphocytes is presented in Table 2,
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Figures 2 and 3. No significant decrease in the proportion of CD3+, CD4+ and CD8+ cells was noted during anti-CD25 antibody therapy. In contrast, CD25+ T cells decreased immediately after antibody infusion and stayed significantly lower for 70 days after transplantation. There was no significant difference between huCD25mAb and daclizumab except at the first time point. A significant decline of CD25+ T cells was achieved after 30 min following intravenous huCD25mAb and daclizumab administration on day 0 (3.40 ± 1.83 to 0.03 ± 0.07 and 1.11 ± 0.87 to 0.00 ± 0.00, respectively). A slight rise was also observed on day 14 before...
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huCD25mAb administration (0.04 ± 0.10) and day 15 (0.14 ± 0.24), and then an immediate drop was observed. The low level of CD3 CD25+ T cells and CD3 CD25+ T cells in huCD25mAb group on day 70 was 0.03 ± 0.05 and 0.31 ± 0.47, respectively.

Correlation between serum anti-CD25 antibody concentration and percentages of lymphocyte subgroups. The concentration-effect plot between serum concentration of huCD25mAb and corresponding lymphocyte subgroups CD3 CD25+ and CD3 CD25+ proportion (%) were shown in Figure 4, by which a significant negative correlation was noted. Thirty minutes after huCD25mAb infusion, the serum huCD25mAb concentration increased to 15.17 ± 3.09 mg·L⁻¹, while the proportion of CD3 CD25+ and CD3 CD25+ T cells decreased to 0.03 ± 0.07 and 0.37 ± 0.49 at the same time, which was less than 10 percent of the baseline (Fig. 4B). The serum huCD25mAb concentration of 5 mg·L⁻¹ seems to be the cutoff point of significant drop in CD3 CD25+ and CD3 CD25+ T cells level, and 1 mg·L⁻¹ level seems to keep saturation for up to 70 days (Fig. 4A). huCD25mAb levels higher than 0.96 ± 0.76 mg·L⁻¹ until 70 days after transplantation were associated with a low proportion of CD3 CD25+ and CD3 CD25+ (0.03 ± 0.05, 0.31 ± 0.47, respectively) on the circulating lymphocytes. Pearson Correlation Coefficient test was performed to assess the correlation between serum huCD25mAb concentration and levels of CD3 CD25+ and CD3 CD25+ T cells on the operation day. A statistically significant negative correlation was found with coefficient factor of -0.972 and -0.989, and p = 0.014, 0.006, respectively.

Anti-antibody antibody and neutralizing anti-antibody antibody. Serum samples on day 20, 42, 56 and 70 were tested for development of anti-huCD25mAb or anti-daclizumab antibodies by antibody-bridge methods. Three samples taken from day 20 showed low titers of anti-antibody antibodies (two in huCD25mAb group and one in daclizumab group). These samples were then analyzed for the existence of neutralizing anti-antibody antibodies by competitive ELISA methods and no neutralizing anti-antibody antibodies were detected.

Safety and efficacy. Both huCD25mAb and daclizumab were well tolerated. No serious adverse events (SAE) were experienced after anti-CD25 antibody iv administration. In the huCD25mAb group, only one patient developed delayed graft function (DGF) and withdrew from the trial two weeks posttransplantation. Meanwhile, one patient in the daclizumab group developed acute rejection and withdrew from the trial three weeks post transplantation.

Discussion

The original anti-CD25 mAb (daclizumab, Zenapax, Roche) regimen routinely used clinically consists of 1 mg·kg⁻¹ intravenous administration, with the first dose given within 12 h before transplantation and a subsequent dose to be followed every two weeks for a total of five doses, in combination with cyclosporine, prednisone and azathioprine. This 5-dose regimen achieved adequate serum daclizumab concentration of 3–5 mg·L⁻¹, and resulted in optimum saturation of the IL-2Rα on the circulating lymphocytes for up to 120 days leading an significant reduction of acute rejection after the transplantation. The addition of Zenapax to the immunosuppressive regimen has resulted in further reductions in the incidence of acute rejection.

In this prospective study, we analyzed the pharmacokinetics and pharmacodynamics of two doses (1 mg·kg⁻¹) of humanized anti-CD25 mAb (huCD25mAb) in patients receiving primary renal transplants and routinely immunosuppressive therapy in combination with triple drugs, CsA, a calcineurin inhibitor, mycophenolate mofetil (MMF) and prednisone. One of the main purposes of the study was to determine whether the serum concentration of huCD25mAb in renal recipients with a 2-dose regimen could blockade interleukin-2 receptor (IL-2R) and provide sufficient therapeutic level for preventing acute graft rejection.
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L·Kg⁻¹ (4.94 ± 1.50 L). The results suggested that huCD25mAb distributed through the vascular space, but had limited penetration into the extravascular space. The \( CL \) of the mAb was 0.204 mL·h⁻¹·Kg⁻¹. Though the steady state could not be reached after administration of two-dose huCD25mAb, the estimates of the AUC for the second dose, AUC₂, were used, and this was considered the best index of exposure to huCD25mAb in our study.

The most important data from our study was the concentration-effect correlation after 2-dose regimen. The concentration-effect plot (Fig. 4) demonstrated that serum level of 5 mg·L⁻¹ was the maximum concentration of humanized anti-CD25 mAb for maintaining immunosuppression by which a lower CD3⁻CD25⁺ and CD3⁺CD25⁺ lymphocyte subgroups could be achieved, and the level of 1 mg·L⁻¹ could keep saturation up to 70 days. The two dose regimen of huCD25mAb used in these patients was shown to be effective in eliminating CD3⁻CD25⁺ and CD3⁺CD25⁺ lymphocyte subgroups for at least 70 days. Therefore, huCD25mAb—may prevent acute rejection effectively because most acute rejections happen during the first six weeks after surgery.

In summary, the results from this study provide evidence that the 2-dose huCD25mAb regimen may be as effective as daclizumab in rapidly achieving a high therapeutic concentration of humanized anti-CD25 mAb and a significant decline of CD3⁻CD25⁺ and CD3⁺CD25⁺ lymphocyte subpopulations lasting approximately 70 days in primary transplant recipients. This two-dose clinical protocol not only supplies preliminary pharmacokinetic (PK) and pharmacodynamic (PD) information for the humanized anti-CD25 mAb but also provides evidence that a two-dose regimen of huCD25mAb may result in effective maintenance of immunosuppression in kidney transplantation recipients. The results suggest that this two-dose protocol may provide a feasible strategy for reducing incidence of acute graft rejection and more choices for recipients following renal transplantation. Further safety and efficacy of huCD25mAb will be investigated in the ongoing Phase II and III clinical trials.

Materials and Methods

Drug nomenclature. HuCD25mAb (Shanghai Key Lab of Cell Engineering and Shanghai National Engineering Research Center for Antibody Medicine, Batch No: 20050601), and daclizumab (Roche Laboratories, Inc., Nutley, NJ, USA. Batch No: B0027) are humanized IgG1 monoclonal antibodies that bind specifically to the alpha subunit (p55 alpha, CD25 or Tac subunit) of the human high-affinity interleukin-2 (IL-2) receptor (IL-2Rα) expressed on the

![Figure 4. The concentration-effect plots display the coherence between huCD25mAb serum concentration and CD25⁺ cell proportion [%] (A). huCD25mAb concentration of about 5 mg·L⁻¹ is to be the cutoff point of significant drop in CD3⁻CD25⁺ and CD3⁺CD25⁺ cells and less than 1 mg·L⁻¹ is to keep saturation for up to 70 days (B). □, huCD25mAb serum concentration (mg·L⁻¹); ○, CD3⁻CD25⁺ proportion(%); ▲, CD3⁺CD25⁺ proportion(%).](image.png)
Surface of activated lymphocytes. Both of two reagents are 25 mg/5 mL/vial, supplied as a clear, sterile, colorless concentrate for further dilution and intravenous administration. In addition to 5 mg of antibody, each milliliter also contains 3.6 mg sodium phosphate monobasic monohydrate, 11 mg sodium phosphate dibasic heptahydrate, 4.6 mg sodium chloride, 0.2 mg polysorbate 80, and may contain hydrochloric acid or sodium hydroxide to adjust the pH to 6.9. No preservatives are added.

Study design and patients. This study was designed as a single-center, open-label clinical trial. As it was a preliminary comparative study instead of a safety and efficacy study, the sample size was not based on statistical considerations. However, the patients who developed presumed acute rejection (clinically diagnosed and resulting in corticosteroid or antilymphocyte therapy) during the study period were described.

Adults (18 yr of age or older) receiving primary renal allografts from cadaveric donors or human leukocyte antigen (HLA)-identical living donors, were eligible for the study. Patients were excluded if they were receiving multiple organ transplants or were previously exposed to IL-2 receptor directed monoclonal antibodies. Patients with other significant diseases or infections were also excluded.

A total of 15 primary kidney transplant recipients were randomized to intravenous administration of either huCD25mAb (n = 10) or daclizumab (n = 5) at a dose of 1 mg·kg⁻¹ pre-transplantation (day 0) and two weeks after the operation (day 14). HuCD25mAb or daclizumab was diluted to a total volume of 50 mL with 0.9% NaCl solution and infused intravenously within 30 min. Besides antibody, the standard triple therapy immunosuppression protocol consisted of cyclosporine, mycophenolate mofetil and steroids. Intraoperatively, 0.5–1 g of methylprednisolone was administered as an intravenous infusion, followed by 0.25–0.5 g per day on days 0 to 3. After reintroduction of oral feeding, treatment was changed to oral therapy with prednisone. This dosage was gradually reduced to a final dose of 5–7.5 mg per day. Treatment with cyclosporine was started on operation day after reperfusion of the transplanted kidney or serum creatinine (Scr) less than 250 μmol·L⁻¹ in a dose of 4–8 mg·kg⁻¹ per day intravenously for ten days and changed into oral administration of 3–5 mg·kg⁻¹ per day. Treatment with mycophenolate mofetil was started on day 3 after operation in a dose of 1.5–2.0 g per day and gradually reduced. Whole blood cyclosporine concentrations were measured by means of an immunoassay specific for the parent compound once a week or more. Doses were adjusted to maintain trough levels of 0.15–0.20 mg·L⁻¹ cyclosporine. To avoid potential interactions between anti-CD25 antibody and other immunosuppressive drugs, use of all other immunosuppressants apart from triple therapy was prohibited during the whole study period, unless acute rejection happened. Biochemical and physical examinations were monitored in pre-transplantation, peri-operative and post-operative period regularly. The follow-up period was 70 days.

The study was approved by State Food and Drug Administration (Clinical trial approval number: 2005L02669) and the ethics committee of Zhongshan Hospital Fudan University (Shanghai, China). The study was then conducted in Zhongshan Hospital in accordance with the revised Declaration of Helsinki and Good Clinical Practice Requirements. All of the patients signed informed consent forms before participating in the study.

Sample collection. Venous blood samples for analysis of lymphocyte subsets and humanized anti-CD25 antibody concentration were drawn on day 0 and 14, before drug administration; 0 and 30 min after discontinuation of the i.v. infusion; then on day 1, 6, 9, 15, 20, 28, 42, 56 and 70 after the renal transplantation for a total of 15 times. In total, 5 mL whole blood was drawn each time. For each sample, serum was harvested by means of centrifugation from 3 mL whole blood and stored for analysis at -20°C. The sample was also used to determine the induction of anti-huCD25mAb or anti-daclizumab antibody and neutralizing anti-antibody antibody. For each lymphocyte subset analysis, 2 mL whole blood sample was collected in anticoagulated tubes, and the samples had to be stained and detected by FCM within 24 h.

ELISA for anti-CD25 antibody levels. Serum concentrations of anti-CD25 antibody were determined by a directed competitive ELISA using anti-CD25 monoclonal antibody in tested samples and a horseradish peroxidase (HRP)-labeled anti-CD25 monoclonal antibody. The HRP-labeled anti-CD25 antibody and a series of calibration standards were provided by Shanghai Key Lab of Cell Engineering and Shanghai National Engineering Research Center for Antibody Medicine. In detail, Nunc Easywash 96-well plates were coated and incubated with CD25/Fc (R&D systems, Category No 1020-RL) at a concentration of 5 mg·L⁻¹ (100 μl/well) or buffer alone at 4°C overnight. The solution was aspirated and the microtiter plates were blocked with 200 μl/well of 1% bovine serum albumin (BSA) in tris buffered saline (TBS). Then the microtiter plates were washed three times with Tris-Buffered Saline Tween-20 (TBST). Anti-CD25 antibody standards, quality control (QC) samples and tested samples were added at 100 μl/well and incubated with HRP-labeled anti-CD25 antibody for 2 h at 37°C. Standard or sample competed with sub-saturated HRP-labeled anti-CD25 antibody to target directly CD25/Fc coated on the plates. After added substrate for coloration, the standard curve was obtained through logistic four-parameter regression, and the serum anti-CD25 antibody concentration was calculated through standard curve. Calibration standards and QC samples were run in quadruplicate and samples were run in duplicate.

Lymphocyte subsets, such as CD3 and CD25, CD4 and CD8, were monitored periodically by flow cytometry. The cells were double-stained with mouse anti CD3-FITC (IgG₁, Becton Dickinson, USA, Category No. 349201) and mouse anti CD25-PE (IgG₁, Becton Dickinson, USA, Category No. 341009) for detection of CD3⁺/CD25⁺ subsets. For analysis of CD4⁺ and CD8⁺ population, a single staining was performed with mouse anti CD4-FITC (IgG₁, Becton Dickinson, USA, Category No. 340133) and mouse anti CD8-FITC (IgG₁, Becton Dickinson, USA, Category No. 347313). Isotype control antibody, fluorescent-conjugated IgG1 and a different anti-CD25 antibody (provided by Shanghai Key Lab of Cell Engineering and Shanghai National Engineering Research Center for Antibody Medicine) were used as negative control. The percentage of circulating lymphocytes with a free CD3 and CD25, CD4, CD8 was reported as mean ± standard deviation (SD) (X ± SD).

To determine anti-antibody antibody in the serum samples on day 20, 42, 56, 70, the antibody-bridge method was used. When the samples were positive, neutralizing anti-antibody antibody would subsequently be detected by competitive ELISA method.
Data analysis. The PK parameters were obtained using the Practical Pharmacokinetic Program 3p97 (Version 97; Chinese Pharmacological Association, Beijing, China). Clearance rate (Cl), volumes of distribution (Vd), elimination half-lives (T1/2e) and areas under the concentration-time curves after the second dose (AUC2) were calculated by statistical moment theory using trapezoidal rule in Micro Excel XP software. Maximum serum concentration values (Cmax) and the corresponding time (Tmax) were defined as observed. Continuous data were presented as mean ± SD, median or range, and categorical data were presented as counts or percentage. Both serum concentrations of huCD25mAb and lymphocyte subsets proportion (%) were summarized over all subjects at each time-point.

Statistical methods. Demographic data, consisting of gender, age, weight and cause of end-stage renal disease (ESRD), was summarized using descriptive statistics. Statistical analyses were performed with a standard computerized statistical program, SPSS 13.0 for Windows software (SPSS, Inc., Chicago, IL, USA). For differences between two groups, if the t-test assumptions were met we used t-test, but if the t-test assumptions were not met we used the Wilcoxon Test. p value <0.05 was considered to be statistically significant.

Acknowledgements

This work was supported by grants from National Natural Science Foundation of China, Ministry of Science & Technology of China (973 and 863 program projects), and Shanghai Commission of Science & Technology (Key Laboratory and Projects).

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