Emerging Therapies:  
Spectrum of Applications of Monoclonal Antibody Therapy 

Thomas A. Waldmann (Chair), Ronald Levy, and Barry S. Coller

This article focuses on the recent dramatic advances in the applications of monoclonal antibody therapy to hematopoietic and neoplastic disease. The increase in the understanding of the role of growth factors and their receptors in the pathogenesis of malignancy and other undesirable hematological events taken in conjunction with the ability to produce humanized chimeric monoclonal antibodies to these targets is providing a new perspective for the treatment of leukemia, lymphoma and breast cancer, autoimmune disease and prevention of ischemic complications. Dr. Waldmann describes approaches targeting the Her2/neu and the IL-2/IL-15 receptor systems. The Her2/neu receptor is overexpressed in select breast, ovarian, gastric and pancreatic neoplasms. The use of trastuzumab (Herceptin) in the treatment of patients with breast cancer whose tumors overexpress this receptor are reviewed. The IL-2 receptor (Tac) is expressed on select malignant cells (adult T cell leukemia, hairy cell leukemia) and activated T cells involved in autoimmune disease and organ rejection. Humanized anti-Tac alone (daclizumab, Zenapax) or armed with toxins or radionuclides have been used successfully in the treatment of leukemia. Dr. Levy updates the experience with rituximab targeting CD20 on B cell lymphomas and reviews the antibodies to CD3, CD22, CD33, CD52, HLA-DR β chain and HLA-D currently in or proposed for clinical trials, including radiolabelled antibodies. In the last section, Dr. Coller reviews the therapeutic results achieved with abciximab (ReoPro), an antagonist of platelet receptor GPIIb/IIIa for the prevention of restenosis in percutaneous coronary interventions and the treatment of unstable angina. The mechanism of action, pharmacology and safety and efficacy of abciximab are reviewed.

INTRODUCTION

Thomas A. Waldmann, M.D.*

The development of monoclonal antibody technology 25 years ago by Köhler and Milstein has provided enormous opportunities for ex vivo diagnosis in a range of disorders.1 However, in the area of immunotherapy of human disease monoclonal antibodies are just beginning to fulfill the promise inherent in their great specificity for recognizing and selectively binding to antigens on cells.2-4

Monoclonal antibodies (mAbs) have been applied clinically to the diagnosis and therapy of cancer and for the modulation of the immune response to produce immunosuppression for the treatment of autoimmune and graft-versus-host disease (GVHD) and for the prevention of allograft rejection. Furthermore, the role of monoclonal antibodies in the treatment of bacterial infections and to inhibit the accumulation of neutrophils and thus reduce tissue damage in bacterial meningitis and myocardial reperfusion injury has been under active study. Finally, monoclonal antibodies have been proposed for the therapy of myocardial infarctions and for the reversal of drug toxicity.

HER-2/neu: Target for Treatment of Epithelial Malignancy

HER-2/neu, also known as c-erb B-2, is expressed on the breast, ovarian, gastric and prostatic tumors of subsets of patients with these disorders.5,9 HER-2/neu, the product of the proto-oncogene c-erb B-2 is a 185 kDa transmembrane receptor with protein tyrosine kinase activity that is a member of the epithelial growth factor (EGF) receptor family. This receptor is modestly expressed in normal adult tissues; however, it is strongly associated with the epithelial solid malignancies and is overexpressed in approximately 25–35% of human gastric, lung, prostatic and breast carcinomas. HER-2/neu overexpression in breast carcinoma is inversely related

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to estrogen receptor expression and is correlated with poor prognosis. Trastuzumab (Herceptin™), an IgG1 mAb that contains human framework constant immunoglobulin regions associated with the complementarity determining regions of the murine antibody (4D5) that binds to HER-2/neu, was shown to enhance the antitumor activity of paclitaxel and doxorubicin when studied in mice bearing HER-2/neu overexpressing human breast cancer xenografts. Two clinical trials supported the efficacy of trastuzumab especially when used in association with chemotherapy. When studied as a single agent in a multicenter open-label single-arm clinical trial, patients with the 2+ level of overexpression of HER-2 (based on a 0-3+ scale) obtained a partial remission in 2% of cases, whereas in patients with 3+ levels of overexpression 2% of treated patients achieved a complete remission and in an additional 15% a partial remission was reported. In a randomized control trial patients with metastatic breast cancer who had not been treated previously with chemotherapy for metastatic disease obtained significantly greater responses (45% vs. 29% (p < 0.001)) and longer median duration of response (9.1 months vs. 5.8 months) when they received trastuzumab and chemotherapy (paclitaxel or cyclophosphamide plus doxorubicin) as compared to those who received chemotherapy alone. On the basis of these data, trastuzumab has received marketing clearance from the FDA for use in the treatment of patients with HER-2 overexpressing breast cancer. Toxicity, especially cardiac toxicity (including death), has been reported in association with the use of trastuzumab, especially in association with a cardiotoxic regimen such as an anthracycline plus cyclophosphamide (28% of patients on trastuzumab plus anthracycline and cyclophosphamide developed cardiac toxicity compared to only 7% of patients on an anthracycline plus cyclophosphamide without trastuzumab).

I. IL-2R and IL-15R: Targets for Immunotherapy of Leukemia/Lymphoma and Autoimmune Disease, and for the Prevention of Organ Allograft Rejection

Thomas A. Waldmann, M.D.

The expression of interleukin-2 (IL-2), the induction of its multisubunit receptor and the subsequent interplay of this ligand with its receptor are pivotal events in T cell activation. Our present understanding of the normal IL-2/IL-2R system and of disorders in this network in disease opens the possibility for more specific immune intervention. The clinical application of agents that inhibit IL-2 function by acting on the IL-2 receptor has provided a new perspective for the prevention of organ allograft rejection, for the treatment of select T cell-mediated autoimmune disorders, and for the therapy of leukemia and lymphoma.

IL-2 is a 15.5 kDa glycoprotein that exerts its effect on activated T cells, natural killer (NK) cells and B cells by binding to a high-affinity form of the IL-2R. This high-affinity IL-2R is composed of three distinct membrane components: the 55 kDa IL-2Rα chain (Tac, CD25), the 70-75 kDa IL-2Rβ chain (CD122), and the 64 kDa common γ chain (CD132). Cytokines such as IL-2 manifest considerable redundancy that is explained by the sharing of common receptor subunits among members of the cytokine receptor family. Most of these cytokines have their own “private” receptor, but IL-2 also shares two of its receptor subunits. In particular, the γ chain is shared by IL-2, IL-4, IL-7, and IL-9. Recently two groups including our own simultaneously reported the recognition of an additional cytokine, IL-15, in this family that employs γc and can stimulate T cell proliferation. In T and NK cells the IL-15 receptor includes IL-2Rβ and γc subunits, which are shared with IL-2 as well as an IL-15-specific receptor subunit, IL-15Rα. IL-2 and IL-15 share many features: they are both members of the 4-helix bundle cytokine family, and they both use IL-2Rβ and γc for their action in T cells. Nevertheless, dramatic differences exist between these two cytokines in terms of their cellular sites of synthesis and the levels of control of their synthesis and secretion. IL-2 is produced by activated T cells, and its expression is controlled at the levels of mRNA transcription and stabilization, whereas the control of IL-15 expression is much more complex with regulation at the levels of transcription, translation and intracellular trafficking and translocation. As predicted from their sharing of receptor subunits, IL-2 and IL-15 have certain redundant functions including their action in innate immunity where they stimulate NK cell development, survival and activation. In contrast, they have unique roles in many of the adaptive immune responses of T cells where their functions are distinct. The unique role of IL-2 is in the maintenance of peripheral tolerance by causing the suicide of self-reactive T cells by a mechanism termed activation-induced cell death (AICD). In contrast, IL-15 inhibits IL-2-mediated AICD and has a predominant role in the maintenance of immunological memory, especially the development and persistence of memory-type CD8 cells directed toward foreign pathogens.

The private receptor for IL-2, the IL-2Rα, has become a target for immune intervention. The scientific basis for this approach is that resting normal cells do not express IL-2Rα, whereas it is expressed by a proportion of the T cells involved in organ allograft rejection, by T cell-mediated autoimmune disease, and by select leukemias and lymphomas. In particular, IL-2Rα is constitutively expressed by the abnormal cells in certain forms of lymphoid neoplasms including human T cell lympho-
tropic virus I (HTLV-I)-induced adult T cell leukemia/lymphoma (ATL), cutaneous T cell lymphoma (CTCL), B-cell hairy cell leukemia, and Hodgkin’s disease.\(^1\)

To exploit this difference in IL-2R\(\alpha\) expression between normal cells and leukemic T cells a series of approaches were developed including those involving unmodified murine antibodies to IL-2R\(\alpha\) (anti-Tac), the humanized form of this antibody (daclizumab, Zenapax), as well these antibodies armed with toxins or \(\alpha\)- and \(\beta\)-emitting radionuclides. The original IL-2R\(\alpha\) studies focused on the treatment of HTLV-I-associated ATL, an aggressive leukemia/lymphoma of mature lymphocytes caused by the retrovirus HTLV-I. No chemotherapeutic regimen appears successful in altering patient survival, and the patients have a median survival duration of only 9 months.\(^6\) The retrovirus HTLV-I encodes a 42-kDa protein termed tax that indirectly stimulates the transcription of numerous host genes including those of IL-2 and IL-2R\(\alpha\) that are involved in T cell activation and potentially HTLV-I-mediated leukemogenesis. The malignant ATL cells constitutively express 10,000–35,000 IL-2R\(\alpha\) chains identified by the anti-Tac mAb, whereas the patient’s normal resting cells do not. Furthermore, in approximately 10–20% of patients with ATL there is evidence supporting an autocrine loop involving IL-2 and IL-2R-dependent expansion of the leukemic cells. These observations led us to perform therapeutic trials with the unmodified murine version of anti-Tac mAb.\(^7\) Six of 19 patients treated developed a partial (4) or complete (2) remission lasting from one month to over 9 years as assessed by phenotypic analysis as well as molecular genetic analysis of HTLV-I proviral integration and T cell receptor gene rearrangements. On the basis of a murine model of ATL we have developed, this action appears in part to be due to the prevention of the interaction of IL-2 with its growth factor receptor leading to cytokine deprivation-mediated apoptotic cell death. Activation-induced cell death also is clearly a factor in this effective therapeutic response. Although murine antibodies such as murine anti-Tac are of value in the therapy of human disease, their effectiveness is limited because such rodent monoclonal antibodies have a short in vivo survival in humans, induce an immune response that neutralizes their therapeutic effect, and may be relatively ineffective at recruiting host effector functions. To address this issue a humanized form of anti-Tac (Hu-anti-Tac) was developed that retained the complementarity-determining regions from the mouse but had virtually all of the remainder of the molecule derived from human IgG1.\(^8\) The humanized version of anti-Tac had improved pharmacokinetics (t\(_{1/2}\) survival of 20 days for the humanized as compared to 40 hours for the murine version), is virtually nonimmunogenic in humans, and functions in antibody-dependent cellular cytotoxicity with human mononuclear cells. Hu-anti-Tac has been used in benign as well as malignant disorders. Following encouraging observations in animal models and in phase I/II trials, Hoffmann-La Roche conducted two double-blind placebo-controlled randomized trials that included 535 evaluated patients to determine the value of Hu-anti-Tac (daclizumab, Zenapax) in preventing renal allograft rejection.\(^9\) In each trial all patients received a standard immunosuppressive regimen. The parallel treatment groups also received either an intravenous placebo or a dose of 1.0 mg/kg of daclizumab prior to transplant and on four subsequent occasions separated by 2 weeks. No drug-specific adverse events or increased morbidity were observed. Acute rejection episodes were reduced by 40% in patients treated with daclizumab (p \(\leq\) 0.01). Ninety-eight percent of the patients receiving triple immunotherapy and daclizumab retained their renal allograft for 6 months whereas only 92% of the patients in the placebo controlled group retained their grafts (p = 0.02). On the basis of these phase III trials the FDA approved daclizumab for use in the prevention of acute kidney transplant rejection. In addition to its use in the prevention of organ allograft rejection, we have shown that daclizumab is of value in the therapy of T cell-mediated autoimmune disorders. In particular, daclizumab provided effective treatment for noninfectious intermediate and posterior uveitis in a clinical trial.\(^10\) Furthermore, daclizumab therapy of patients with the neurological disease HTLV-I-associated myelopathy (tropical spastic paraparesis) led to a reduction in HTLV-I viral load, a decrease in the spontaneous T cell proliferation ex vivo of the peripheral blood mononuclear cells of patients, and a stabilization or amelioration of the neurological disease.\(^11\)

A limitation in the use of unmodified monoclonal antibodies in the treatment of leukemia/lymphoma is that they are relatively ineffective as cytocidal agents. This is true in late-stage HTLV-I-associated adult T cell leukemia, a stage when the cells continue to express IL-2R\(\alpha\) but no longer produce nor require IL-2 for their proliferation. This limited efficacy of many unmodified mAbs in cancer therapy led to the alternative approach of using agents such as anti-Tac as carriers of cytotoxic substances including toxins or \(\alpha\)- and \(\beta\)-emitting radionuclides. In one group of studies Kreitman, Pastan and coworkers developed LMB2, a version of anti-Tac linked to a truncated form of a Pseudomonas exotoxin (PE38) that has a deletion of domain 1. Domain 1 is responsible for unwanted ubiquitous binding of the toxin. A single chain toxin fusion protein, LMB2 (anti-Tac Fv-PE38) in which the variable region (Fv) of anti-Tac was joined in peptide linkage to PE38, has proved to be very effective in the treatment of IL-2R\(\alpha\)-expressing hairy cell leukemia with all four treated patients undergoing a partial or complete remission.\(^12\) In an alternative approach radiolabeled mono-
clonal antibodies were developed to deliver a cytotoxic agent to target leukemic cells. One advantage in the use of radiolabeled antibody conjugates is that with the appropriate choice of radionuclide, radiolabeled monoclonal antibodies kill cells at distances of several cell diameters. Therefore, a radiolabeled mAb such as anti-Tac binding to an IL-2R-expressing cell may kill adjacent nonexpressing cells, thereby overcoming the tumor cell antigenic heterogeneity that presents a problem for most other monoclonal antibody-mediated approaches. In a clinical trial involving 90Y-anti-Tac for the therapy of HTLV-I-associated ATL, in 16 evaluable patients seven partial and two complete remissions were observed. Clinically meaningful (i.e. ≥ Grade 3) toxicity was limited to the hematopoietic system. An important element in our present developmental efforts with systemic radioimmunoimmunotherapy involves the use of α-emitting radionuclides to arm humanized anti-Tac or its fragments. Alpha-emitting radionuclides that are under evaluation include 212Bi, 213Bi, and 211At.

Although IL-2Rα-directed therapy has met with considerable success in selected leukemias, autoimmune disorders and allograft rejection, approaches directed toward this receptor subunit have limitations. In particular, antibodies to IL-2Rα do not inhibit the actions of IL-15, a cytokine that does not bind to this subunit. Abnormalities of IL-15 expression have been described in patients with rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, chronic liver disease due to hepatitis C, T cell alveolitis, and in diseases associated with the retroviruses HIV and HTLV-I. Thus new approaches directed toward IL-15, its receptor or its signaling pathway may be of value in the therapy of these disorders. Our own IL-15-directed therapeutic approach has focused on the cytokine receptor subunit and signaling pathways shared among multiple cytokines including IL-15 in an effort to yield more profound immunosuppression than can be achieved by the inhibition of the action of a single cytokine such as IL-2. Our initial trials use Mikβ1, an antibody directed toward the β subunit that is shared by IL-2 and IL-15. The humanized version of this antibody inhibits the action of IL-15 on T and NK cells and prolongs renal allograft survival in cynomologous monkeys. In our current clinical trial we are evaluating Mikβ1 in the therapy of patients with T cell-type large granular lymphocytic leukemia associated with granulocytopenia. The monoclonal large granular lymphocytosis involved in this disease express IL-2/IL-15Rβ and γc, but not IL-2Rα.

In summary, our expanding understanding of the IL-2/IL-2R as well as IL-15/IL-15R systems taken in conjunction with the ability to produce humanized antibodies directed toward the IL-2 and IL-15 receptors have provided a new perspective for the prevention of organ allograft rejection for the treatment of select autoimmune disorders and for the therapy of those leukemia/lymphomas that express the IL-2 receptor.

II. MONOCLONAL ANTIBODIES FOR THE TREATMENT OF LYMPHOMA: MANY DIFFERENT WAYS TO USE A NEW MODALITY

Ronald Levy, M.D.*

Monoclonal antibodies are finally finding their way into the standard practice of hematology and oncology. With the approval by the FDA of rituximab for the treatment of relapsed low-grade lymphoma, a new and welcome modality has been added to the therapeutic armamentarium. Several other monoclonal antibodies are in late stages of clinical development and are likely to be available to the practicing hematologist/oncologist in the near future. In this section, the current states of naked monoclonal antibodies, antibody-drug conjugates and radiolabeled antibodies mainly for the treatment of non-Hodgkin’s lymphoma (NHL) are reviewed.

Rituximab

Rituximab (Rituxan) is a chimeric human/mouse antibody directed against the CD20 antigen, which is expressed on normal and malignant B-lymphocytes. In the original phase I trial of this agent it was apparent that it had anti-tumor activity with no dose-limiting toxicity. In the pivotal trial that led to the FDA approval for patients with recurrent low-grade lymphoma, the response rate of such patients was approximately 50% PR + CR with a median duration of these responses of approximately 13 months. Now that this drug is in widespread use, a number of questions are being addressed:

- How to use the drug most effectively
- When to use the drug most optimally
- What range of lymphomas to treat
- How the drug works

The standard dosing schedule of 375 mg/m² weekly x 4 has been investigated and compared to longer dosing regimens and repetitive dosing regimens. Because of the pharmacokinetics of this chimeric human/mouse antibody, detectable levels of the antibody are present as long as several months after the standard 4-week dose regimen. Therefore it is no surprise that extended numbers of doses have not led to substantial increases in response rates. The question of repetitive dosing cycles at more prolonged intervals is still open and under investigation.

It is clear that patients who initially respond to rituximab can be retreated successfully at the time of relapse. Approximately 40% of previously sensitive patients

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Mechanism of Action
The evidence to date suggests that the drug has its primary mechanism of anti-tumor action by binding to the tumor cells and attracting killer cells through an antibody-dependent cellular cytotoxicity mechanism (ADCC). Experimental studies have shown that cells bearing an Fc receptor are necessary for the acute anti-tumor effect of rituximab. These studies, conducted in special gene knock-out mouse models, point the way for second generation antibody products, those with optimized Fc fragments that may interact more effectively with Fc receptors. The longer term anti-tumor effects of the drug and the longer durations of second remissions induced by this drug are more difficult to understand by an ADCC mechanism. One hypothesis involves a potential active immune response by the host against his own tumor to account for these later effects, but there is as yet no evidence for or against this hypothesis.

Tables 1 and 2 describe trials that are currently active or are about to be activated by cooperative groups. Table 3 lists other clinical trials underway sponsored by Genentech. Additional studies are underway in mantle cell lymphoma, multiple myeloma, post-transplant lymphoproliferative disease, CLL, Waldenström’s macroglobulinemia, hairy cell leukemia, acute lymphocytic leukemia, and autoimmune diseases.

Other Antibodies in Clinical Trials (Table 4)

**Campath-1H**
Campath-1H is a humanized version of rat monoclonal antibody directed against the CD52 antigen which is found on all mononuclear leukocytes. Clinical trials using Campath-1H have been performed over the past 10 years in multiple disease settings, including non-Hodgkin’s lymphoma, chronic lymphocytic leukemia, multiple sclerosis, and other autoimmune diseases, solid organ and bone marrow transplants, and GVHD. This
drug is associated with acute infusion reactions (mediated by tumor necrosis factor [TNF], interleukin-6 [IL-6], and interferon-γ), prolonged T cell depletion and opportunistic infections. These complications can be controlled by gradual escalation of initial doses and prophylactic treatment with anti-inflammatory and anti-infectious medications.

Three separate phase II studies have been performed on patients with NHL and CLL. The most recent study involved patients with B-cell CLL who had received prior alkylating agents and fludarabine therapy. Ninety-three patients were enrolled in 22 study centers. There was a substantial objective response rate of 33% with resolution of peripheral lymphocytosis in 98% of evaluable patients. The median duration of response has not yet been reached at greater than 7 months of follow-up. Because of the immunosuppressive effects of this monoclonal antibody, serious adverse events occurred that involved infectious complications. This drug is currently under FDA review for the treatment of advanced-stage fludarabine-refractory CLL patients.

**hL2 (Epratuzumab)**

This humanized murine antibody is directed against the CD22 antigen present on normal and malignant B lymphocytes. A phase I/II dose escalation trial has been completed in patients with relapsed NHL. A maximum dose of 1000 mg/m² given weekly for 4 weeks was reached. Tumor responses were seen, no dose-limiting toxicity was observed, and there was no evidence of immunogenicity.

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**Table 2. Currently approved but not yet activated NCI-sponsored trials of rituximab.**

<table>
<thead>
<tr>
<th>Institution</th>
<th>Trial Description</th>
</tr>
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<tbody>
<tr>
<td>NCI</td>
<td>Dose adjusted EPOCH chemotherapy with rituximab in previously untreated aggressive non-Hodgkin's lymphoma</td>
</tr>
<tr>
<td>SWOG</td>
<td>A randomized phase III trial of ESHAP chemotherapy with or without rituximab for the treatment of relapsed or refractory CD20+ aggressive B cell non-Hodgkin’s lymphoma</td>
</tr>
<tr>
<td>CALGB</td>
<td>Interleukin-2 and anti-CD20 in CD20+ B cell lymphoid malignancies</td>
</tr>
<tr>
<td>CALGB</td>
<td>Randomized phase III trial of fludarabine/mitoxantrone/dexamethasone (FMD) with concurrent or subsequent administration of rituximab monoclonal antibody</td>
</tr>
<tr>
<td>ECOG</td>
<td>Rituximab for Waldenström’s macroglobulinemia, a phase II pilot study for untreated and previously treated patients</td>
</tr>
</tbody>
</table>

**Table 3. Other clinical trials of rituximab currently underway.**

**Low Grade Lymphoma**

- In newly diagnosed patients: treatment as a single agent, and in combination with a variety of chemotherapy regimens
- In relapsed disease: as repetitive courses or in combination with a variety of lymphokines and cytokines
- In adjunct to BMT: either before, during, or after the procedure

**Intermediate and High Grade Lymphoma**

- In newly diagnosed patients: in combination with standard chemotherapy regimens
- In relapsed and refractory patients: in combination with a variety of chemotherapy regimens
- As an adjunct to BMT: either before, during, or after the transplant procedure

**Table 4. Other antibodies in clinical trials.**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Characteristics</th>
<th>Target</th>
<th>Cells</th>
<th>Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campath-1H</td>
<td>Humanized rat Mab</td>
<td>CD52</td>
<td>All mononuclear cells</td>
<td>CLL, PLL, NHL</td>
</tr>
<tr>
<td>HLL2 (epratuzumab)</td>
<td>Humanized murine Mab, also radiolabelled</td>
<td>CD22</td>
<td>Mature B cells</td>
<td>B-NHL</td>
</tr>
<tr>
<td>Hu1D10</td>
<td>Humanized murine IgG1 Mab</td>
<td>HLA-DR β chain</td>
<td>B cells, macrophages, dendritic cells</td>
<td>B-NHL</td>
</tr>
<tr>
<td>HuM291</td>
<td>Humanized murine Mab</td>
<td>CD3</td>
<td>Mature T cells</td>
<td>Psoriasis, Renal transplant, T-NHL</td>
</tr>
<tr>
<td>Gemtuzumab (Mylotarg)</td>
<td>Humanized murine Mab-drug (calicheamycin conjugate)</td>
<td>CD33</td>
<td>Myeloid cells</td>
<td>AML</td>
</tr>
<tr>
<td>131I-tositumomab (Bexxar)</td>
<td>Murine Mab radiolabelled with 131I</td>
<td>CD20</td>
<td>Mature B cells</td>
<td>B-NHL,</td>
</tr>
<tr>
<td>IDEC-Y2B8 (Zevalin)</td>
<td>Murine Mab radio-labelled with 90Y</td>
<td>CD20</td>
<td>Mature B cells</td>
<td>B-NHL</td>
</tr>
<tr>
<td>Lym-1</td>
<td>Murine Mab radio-labelled with 131I or 67Cu</td>
<td>HLA-D</td>
<td>B cells, macrophages, dendritic cells</td>
<td>B-NHL</td>
</tr>
</tbody>
</table>

Abbreviations: Mab, monoclonal antibody; CLL, chronic lymphocytic leukemia; PLL, prolymphocytic leukemia; NHL, non-Hodgkin’s lymphoma, AML, acute myeloid leukemia.
This agent is in further clinical testing for the treatment of NHL.\textsuperscript{16}

\textit{Hu1D10}

1D10 is a murine IgG1 monoclonal antibody that binds to a variant of the HLA-DR beta-chain. The antibody was humanized by CDR grafting. The 1D10 antigen is expressed on lymphocytes, macrophages, and dendritic (mesenchymal) cells and on most B cell neoplasms. In pre-clinical studies, 1D10 antigen-positive animals demonstrated a decrease in circulating B cells following infusion of Hu1D10. The Cancer Treatment and Evaluation Program (CTEP) is currently sponsoring a phase I dose escalation study of Hu1D10 in patients with relapsed NHL. At each of five dose levels, ranging from 0.15 mg/kg to 15 mg/kg, patients receive a series of 4 infusions over 2 hours on days 1, 8, 15, and 22. Dose level 3 (1.5 mg/kg) has been reached with preliminary evidence of efficacy and no unexpected or dose-limiting toxicity observed.\textsuperscript{17}

\textit{HuM291}

HuM291 is a humanized murine IgG2 monoclonal antibody against the human CD3 marker. This antibody has undergone preclinical and clinical testing for various indications, where it was noted to profoundly deplete normal circulating T cells. It is now under consideration for clinical testing against CD3-positive T cell lymphomas. In chimpanzee studies, HuM291 induced complete peripheral blood T cell depletion as early as 6 hours after its administration at doses ranging from 0.1 to 10 mg (total dose) per 35–50 kg animal. T cell counts increased as the HuM291 concentration in circulation declined, indicating that the T cell depletion in these animals was reversible.

A multiple dose escalation study of HuM291 given intravenously to renal transplant patients experiencing acute rejection has entered 14 patients at 5 dose levels (0.0015 mg/kg, 0.0045 mg/kg, 0.015 mg/kg, 0.030 mg/kg, or 0.045 mg/kg). Profound T cell depletion was achieved and the extent and duration of T cell depletion appeared to be dose dependent, with the highest dose leading to 14–28 days of T cell counts below 100 cells/mm\textsuperscript{3}. A multicenter study evaluating the safety and pharmacology of HuM291 administered as a single subcutaneous injection to patients with mild or moderate psoriasis has just begun.

CTEP proposes to initiate evaluation of HuM291 in CD3+ T cell malignancies. An initial phase I dose escalation trial would be designed to identify maximal biologic effect with saturation kinetics. Dosing will start at the highest dose given previously on a multi-dose basis, with the dosing interval to be defined by pharmacokinetics studies from ongoing trials. Subjects will be followed for toxicity, T cell depletion and tumor response.

\textit{Antibody-drug conjugate (gemtuzumab; Mylotarg)}

Mylotarg is composed of a humanized antibody against the CD33 antigen that has been chemically conjugated to calicheamicin, a small molecule that kills dividing cells by DNA intercalation. The CD33 antigen is expressed on myeloid progenitors and committed precursors but not on myeloid stem cells. It is expressed on virtually all acute myelogenous leukemia (AML) cells. It has recently been approved by the FDA for the treatment of recurrent AML in the elderly. The antibody-drug conjugate has been shown to be well tolerated, to saturate target sites and to induce a 26\% overall remission rate in patients 60 years and older with CD33 positive AML in first relapse. It causes myelosuppression but does not ablate normal hematopoiesis.\textsuperscript{18}

\textit{Radiolabeled monoclonal antibodies (Bexxar, Zevalin, Lym-1)}

Two radiolabeled antibody products are in advanced stages of clinical development, \textsuperscript{131}I-tositumomab (Bexxar) and IDEC-Y2B8 (Zevalin).\textsuperscript{19,20} Both of these antibodies are murine monoclonal antibodies. Both are directed against the CD20 antigen present on normal and malignant B lymphocytes. Bexxar is labeled with \textsuperscript{131}I and Zevalin is labeled with \textsuperscript{90}Y. Both have shown impressive anti-lymphoma activity, superior to that found with unlabeled monoclonal anti-CD20 antibodies.\textsuperscript{21} Both of these radiolabeled products, however, have dose-limiting toxicity, predominantly bone marrow suppression. As with unlabeled antibodies, studies are underway with these radiolabeled antibodies to determine the optimal timing, range of diseases that can be treated, and strategies of integration with other forms of anti-lymphoma therapy. For instance, the Bexxar product has been studied as primary therapy for patients with low-grade follicular lymphoma.\textsuperscript{22} These studies have shown an impressive response rate. Follow-up studies are under way to determine the duration of these remissions and the tolerance of these patients to subsequent therapies. In another example, the Bexxar product has been studied in conjunction with myeloablative therapy supported by stem cell transplantation.\textsuperscript{23} Long-term remissions of disease have been documented, and studies are underway to interdigitate high-dose Bexxar with conditioning regimens for peripheral stem cell transplantation.

Lym-1 is a murine antibody that recognizes one of the HLA-D antigens that has been radiolabeled with \textsuperscript{131}I or with \textsuperscript{67}Cu.\textsuperscript{24} Both of these have shown clinical activity in patients with lymphoma and CLL with acceptable toxicity profiles. Similar studies have been performed with an \textsuperscript{131}I labeled version of the hLL2 anti-CD22 antibody.
**III. Therapeutic Results with Abciximab, an Antagonist of the Platelet GPIIb/IIIa (αIIbβ3) Receptor**

**Barry S. Coller, M.D.**

Abciximab (Chimeric 7E3 Fab; ReoPro), the Fab fragment of the mouse human chimeric antibody 7E3, which inhibits ligand binding to the platelet GPIIb/IIIa receptor, the αVβ3 receptor, and one or more activated conformations of the αMβ2 receptor, was approved for human use as adjunctive therapy to prevent ischemic complications of percutaneous coronary interventions in December 1994. In the subsequent 5 years, it has been administered to nearly 1,000,000 patients worldwide. Thus, considerable information has been accumulated about its pharmacology, safety, and efficacy. Since it is perhaps the first rationally designed antiplatelet agent and the first integrin receptor antagonist, the experience gained with it may also be of more general interest to those involved in drug development, antiplatelet therapy, and integrin receptor biology. Two low molecular weight GPIIb/IIIa-specific antagonists (eptifibatide and tirofiban), which are modeled on the cell recognition sequence arginine-glycine-aspartic acid (RGD) found in several GPIIb/IIIa ligands, have more recently been approved for human use for indications similar to those for abciximab. Given the limitations of space, the following discussion will focus on abciximab, but recent reviews of these agents are available.

**Pharmacokinetics**

Abciximab binds with high affinity (~1-5 nM) to both GPIIb/IIIa and αVβ3. After the administration of the recommended bolus dose of 0.25 mg/kg, approximately two-thirds of the drug rapidly binds to platelets, resulting in blockade of ≥80% of the GPIIb/IIIa receptors and ≥80% inhibition of platelet aggregation in response to ADP (5–20 µM) in a large majority of patients. Similarly, treatment with the recommended infusion of 0.125 µg/kg/min for 12 hours will sustain ≥80% receptor blockade in the majority of patients, but there is more variability in this response than there is after the bolus dose. The plasma level of unbound abciximab drops to low levels very rapidly after administration. Some abciximab becomes internalized after administration and can be detected on α-granule membranes. Ex vivo mixing studies, as well as in vivo studies, demonstrate that abciximab continually redistributes from one platelet to another over a period of minutes to hours. Abciximab is not excreted in significant amounts in the urine; its major mechanism of catabolism is probably via degradation of platelet-bound antibody at the time senescent platelets leave the circulation. Abciximab does not normally significantly shorten platelet survival. Although it does bind to megakaryocytes, it does not appear to affect megakaryopoiesis.

These pharmacokinetic data have a number of important practical implications:

1) Patients with severe thrombocytosis need a higher dose of abciximab to achieve high grade GPIIb/IIIa receptor blockade because there are too few abciximab molecules in the recommended bolus dose.

2) Platelet-associated abciximab decreases gradually after stopping the drug. In fact, small amounts of platelet-associated abciximab persist in the circulation for a period of two weeks or more after treatment (i.e. longer than the survival of any of the platelets present at the time when the drug was administered), because of redistribution to new platelets entering the circulation.

3) The amount of unbound plasma abciximab available to inhibit transfused platelets is very small, even if the transfusion is given shortly after the abciximab is administered. Thus, platelet transfusions are able to rapidly reverse the drug’s inhibitory effects in nonhuman primates and, presumably, in humans as well. However, since the abciximab on the circulating platelets will redistribute to the transfused platelets, it may be necessary to administer larger numbers of platelets than are traditionally administered to thrombocytopenic patients, especially if the abciximab was recently administered or there is a large pool of platelets, as for example, with splenomegaly. Since heterozygotes for Glanzmann’s thrombasthenia, who have ~50–60% of the normal number of GPIIb/IIIa receptors, do not have excess bleeding, there is reason to believe that hemostasis is relatively intact when the receptor blockade decreases below ~50%. Thus, to rapidly reverse the effect of abciximab soon after its administration, it may be necessary to transfuse nearly as many platelets as are present in the patient’s circulation and spleen (approximately 20 units of platelets in a 70 kg person with a platelet count of 250,000/µL).
FDA Approved Indications
Abciximab has been approved by the FDA for the following indications:

1) As adjunctive therapy of percutaneous coronary interventions (PCI). In double-blind, randomized, placebo-controlled, phase III studies of patients undergoing PCI (including balloon angioplasty, rotational atherectomy, and stent insertion), abciximab administered (in conjunction with aspirin and heparin) as a bolus (0.25 mg/kg) before the procedure followed immediately thereafter by a 12 hour infusion (0.125 µg/kg/min) has been demonstrated to decrease the relative risk of suffering an ischemic complication (death, myocardial infarction, or need for urgent repeat revascularization) by 35–57% (absolute risk reductions of 4.5–6.5%) during the first 30 days.\(^5,17\) Table 5 contains a comparison of the number of vascular events averted by treating 1,000 patients with aspirin or other antiplatelet agents for variable periods of time, stratified for indication, compared to adding a bolus + 12-hour infusion of abciximab before PCI and stent placement in the most recent phase III study (EPISTENT).\(^18\) Moreover, abciximab treatment also significantly decreased overall mortality at 1 year in treated patients undergoing stent placement by 67% (0.8% for patients receiving stents + abciximab vs 2.4% for patients treated with stents, p = .015 for treated patients).\(^19\) In general, diabetic patients do not derive as much benefit from PCI as non-diabetic patients, but abciximab treatment improves the outcomes for diabetics. Thus, the 1-year mortality data for the subset of diabetic patients in EPISTENT were 1.2% for stent + abciximab compared to 4.1% for stent alone (p = 0.011),\(^20\) and pooled data from the EPIC, EPIDLOG, and EPISTENT studies demonstrated a similar mortality advantage for diabetic patients treated with abciximab.\(^21\)

2) As adjunctive therapy for patients with refractory unstable angina who are candidates for PCI. Based on positive results from the CAPTURE study, abciximab is also indicated as treatment for patients with refractory unstable angina who are candidates for PCI and who are expected to undergo PCI within 18–24 hours after starting abciximab.\(^22\) However, just before this chapter went to press, the preliminary results of the GUSTO-4 trial were presented at the 22nd Congress of the European Society of Cardiology.\(^23\) In this study of 7,800 patients believed to have unstable angina, all of whom were treated with aspirin and heparin, adding abciximab treatment for 24 or 48 hours did not decrease the 30-day rates of death or myocardial infarction, and there was a negative trend with abciximab treatment that was worse with the longer infusion time (placebo 8.0%; 24-hour abciximab 8.2%; 48-hour abciximab 9.1%). The reason(s) for the apparently discrepant results between CAPTURE and GUSTO-4 are unclear, but patient selection may be important since CAPTURE was confined to patients who were already refractory to aspirin and heparin, and who had a lesion amenable to PCI.

Cost Effectiveness Ratio
Since abciximab treatment in EPISTENT resulted in a mortality advantage at 1 year, it was possible to assess the cost (in dollars) of saving one year of a patient’s life (i.e., a life-year).\(^19\) The cost per life-year saved by adding abciximab to stent insertion is ~$6,200, which can be compared with coronary bypass surgery for left mainstem coronary artery disease (~$7,000), treatment of acute myocardial infarction with tissue plasminogen activator (t-PA) rather than streptokinase (~$33,000), hemodialysis (~$35,000), and primary prevention of vascular disease by lowering cholesterol with a statin compared to diet (~$54,000-$140,000).\(^19,23\) It also compares favorably with public health measures, such as automobile seat belts (~$150,000) and automobile air bags (~$1,700,000).\(^24\)

Indications Currently Under Investigation
In a randomized, double-blind, phase II study of patients with myocardial infarction receiving aspirin and heparin, a combination of abciximab and 50 mg t-PA (which is half the usual dose) was found to result in a higher frequency of complete blood flow restoration in the affected blood vessel at 60 and 90 minutes after drug administration than treatment with 100 mg of t-PA alone.\(^25\) Abciximab treatment without a thrombolytic agent resulted in reperfusion rates similar to those produced by streptokinase alone. Larger studies using combinations of abciximab and thrombolytic agents are currently underway. Abciximab has also been used in combination with thrombolytic agents in peripheral arterial disease.\(^26\)

Table 5. Comparison of Abciximab treatment for percutaneous coronary intervention (PCI) with other antiplatelet therapies.

<table>
<thead>
<tr>
<th>Entry Condition</th>
<th>Treatment Duration (mo.)</th>
<th>Number Benefitted per 1,000 Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute MI</td>
<td>1</td>
<td>38±5</td>
</tr>
<tr>
<td>Prior MI*</td>
<td>27</td>
<td>36±6</td>
</tr>
<tr>
<td>Primary Prevention*</td>
<td>62</td>
<td>4±3</td>
</tr>
<tr>
<td>PCI** (Abciximab)</td>
<td>0.017</td>
<td>58</td>
</tr>
</tbody>
</table>

* Data from Antiplatelet Trials’ Collaboration (Br Med J 308:1540, 1994) Other antiplatelet therapies included primarily aspirin alone or in combination with dipyridamole. Other regimens included ticlopidine, sulphinpyrazone, dipyridamole, aspirin and sulphinpyrazone, and sulcloxidil. Data include results from 145 trials involving approximately 100,000 patients.

** Data from EPISTENT: 30-day event rates (Lancet 352:87, 1998)

Abbreviations: PCI, percutaneous coronary intervention.
In a placebo-controlled phase II study of 74 patients with acute, nonhemorrhagic stroke, treatment with varying doses of abciximab between 3–24 hours after stroke onset resulted in a trend toward improved clinical outcome at 3 months. There were no major intracranial hemorrhages in the abciximab-treated patients and no increase in symptomatic parenchymal hemorrhages, but there was an increase in asymptomatic intracranial hemorrhages detected by unscheduled CT scans.

There have been anecdotal reports of beneficial effects of abciximab treatment as adjunctive therapy of cerebral angioplasty and stent placement. Patients with a history of heparin-induced thrombocytopenia have undergone TCI with abciximab and an alternative anticoagulant. In addition, abciximab has been reported to be beneficial in treating patients with Kawasaki disease and large aneurysms and thrombi.

Safety Considerations

Thrombocytopenia

Abciximab can induce both pseudothrombocytopenia and true thrombocytopenia. The development of EDTA-dependent pseudothrombocytopenia does not constitute an indication to stop abciximab therapy. Thus, it is vital that thrombocytopenia reported by automated platelet counters be confirmed by analyzing the blood smear for the presence of platelet clumping and, if necessary, by repeating the platelet count with citrate as the anticoagulant. Since abciximab is given in conjunction with heparin, it is necessary to also consider heparin-induced thrombocytopenia in the differential diagnosis. True thrombocytopenia occurs in several percent of patients treated with abciximab, and acute, profound thrombocytopenia (platelet count < 20,000/µl within one day of administration) occurs in between 0.5–1.0% of patients receiving abciximab for the first time. In patients who develop acute, profound thrombocytopenia, platelet counts obtained between 2–4 hours after drug administration nearly always demonstrate significant decreases from baseline values, so it is crucial that a platelet count be performed during that time period so that the drug infusion can be stopped and, if necessary, other measures instituted. Most patients with severe thrombocytopenia respond well to platelet transfusions, and platelet count recovery generally starts within 1–2 days; recovery usually occurs within 5 days, but can take up to 12 days. The mechanism(s) of abciximab-induced thrombocytopenia has not been conclusively established, but there are data supporting an immune mechanism in some cases, perhaps involving the presence of preformed antibodies in the recipient to neoantigen(s) expressed on GPIIb/IIIa as a result of the binding of abciximab. Thrombocytopenia that occurs after readministration of abciximab may be associated with higher titers of antibodies. Platelet activation by abciximab has been proposed as an alternative mechanism for thrombocytopenia, but a number of laboratories have not been able to confirm abciximab-induced platelet activation.

Bleeding

In the first phase III study (EPIC), abciximab treatment significantly increased the risk of major bleeding. Relatively high doses of heparin were used in this study, however, and in the subsequent EPILOG and EPISTENT studies, in which the heparin dose was reduced and weight adjusted, there were no significant increases in major bleeding. Pulmonary hemorrhage has been reported as a serious complication of abciximab treatment and may be difficult to differentiate from other causes of pulmonary compromise. Reported data are inconsistent with regard to whether abciximab treatment increases the risk of hemorrhage associated with cardiopulmonary bypass surgery. There does not appear to be a consensus among cardiovascular anesthesiologists and surgeons regarding the desirability of preoperative and/or postoperative platelet transfusions. If hemostasis cannot be secured, however, in view of the pharmacokinetics of abciximab, it may be appropriate to transfuse a larger number of platelets than is traditionally given to patients undergoing cardiopulmonary bypass surgery.

Immune reactions and results with readministration

Approximately 6% of patients receiving abciximab develop human antichimeric antibody responses (HACA). The titers, in general, are low and decline over a 6-month period. A study of 164 patients who received abciximab for PCI on two separate occasions separated by 1–570 days found that readministration was associated with high procedural (> 99%) and clinical (94%) success, no allergic or anaphylactic reactions, and a risk of acute profound thrombocytopenia of 3% (compared to 0% with first administration). Analysis of the data according to the time interval between the first and second treatment suggested that the risk of developing thrombocytopenia is greater with short intervals between doses.

Restenosis

Results from the EPIC study suggested that abciximab treatment decreased the risk of developing clinically significant restenosis after 6 months. A number of subsequent studies, however, did not support this finding. In the most recent study (EPISTENT), abciximab treatment of patients receiving stents significantly decreased the risk of developing both clinical and angiographic evidence of restenosis in patients with diabetes at 6 months (8.1% vs 16.6% target vessel revascularization) and 1 year (14% vs 22%), but had little effect in non-diabetic patients.
Mechanisms of Action
Abciximab’s primary mechanism of action involves blockade of GPIIb/IIIa receptors. This decreases platelet thrombus formation and thus decreases the risk of vasooclusion at the site of PCI. Blockade of GPIIb/IIIa receptors by abciximab has additional effects, however, that may also contribute to the benefits. Thus, abciximab can decrease platelet-mediated thrombin generation induced by tissue factor and can prolong the activated clotting time (ACT), indicating a potential anticoagulant effect. Abciximab also decreases thrombin-induced platelet microparticle formation, and microparticles have been implicated in supporting thrombin generation and activating endothelial cells. Abciximab has a number of effects that can facilitate thrombolysis (reviewed in [50 and 51]), including 1) increasing clot porosity as a result of preventing clot retraction, 2) decreasing thrombin-mediated activation of a carboxypeptidase (thrombin-activated fibrinolysis inhibitor, TAFI) that inhibits thrombolysis, 3) decreasing release from platelets of the fibrinolytic inhibitors plasminogen activator inhibitor-1 (PAI-1) and α-2 plasmin inhibitor, and 4) decreasing factor XIIa-mediated crosslinking of fibrin.

Some of abciximab’s beneficial effects may operate not only at the site of vascular injury or plaque rupture, but also at the level of the microvasculature distal to the site of vascular injury or plaque rupture. Thus, abciximab may reduce distal embolization of platelet aggregates and platelet-leukocyte aggregates, as well as discharge into the distal circulation of thrombin formed on the platelet surface and the products of platelet synthesis and/or release (ADP, serotonin, vascular endothelial growth factor [VEGF], thromboxane A₂, platelet microparticles); all of these cellular and humoral elements may damage the microcirculation acutely and/or chronically. Experimental data, in fact, demonstrate that abciximab treatment has a cardioprotective effect in an isolated perfused heart model of ischemia and reperfusion.

One intriguing hypothesis is that the long-term mortality benefit found with short-term abciximab treatment is due to the prevention of acute damage to the microvasculature, which in turn, prevents the development of fibrotic foci in the myocardium that may ultimately result in electrical instability. The strong correlation between myocardial enzyme elevations at the time of PCI and long-term mortality risk found in many, but not all, studies are consistent with this hypothesis.

Clinical data supporting a beneficial effect of abciximab treatment on the microcirculation include improved peak blood flow and myocardial contractility 2 weeks after stent placement in patients with myocardial infarctions, faster ST-segment resolution after thrombolytic therapy for myocardial infarction, and prevention of myocardial perfusion abnormalities after rotational atherectomy. Recent evidence suggests that markers of systemic inflammation correlate with cardiovascular risk and so it is particularly interesting that PCI and myocardial infarction are associated with increased surface expression of the leukocyte integrin αMβ2; abciximab not only prevents this increase in αMβ2 expression, but actually decreases expression below baseline values. One potentially important link between platelet deposition and a systemic inflammatory state is the expression of CD40 ligand on the surface of activated platelets, since CD40 ligand (CD154) is a potent activator of the immune response, and perhaps endothelial cells. Patients with acute coronary syndromes have increased circulating levels of soluble CD40 ligand, which probably derives from platelets. It is intriguing to speculate, therefore, that platelet activation due to vascular injury may actually initiate systemic inflammation.

The crossreactivity of abciximab with the integrin αVβ3, which is present on many different cell types including endothelial cells, smooth muscle cells, and platelets, has been suggested to contribute to its beneficial effects since blockade of αVβ3 may decrease both platelet adhesion to osteopontin (a component of atherosclerotic plaque) and platelet-mediated thrombin generation.

In addition, animal model data implicate αVβ3 in intimal hyperplasia after vascular injury (reviewed in [1]). To date, however, there are no data indicating a direct role for αVβ3 blockade by abciximab in its effects. Similarly, although in vitro and ex vivo studies demonstrate the ability of abciximab to bind to an activated conformation of leukocyte αMβ2, there are no direct data linking this reactivity with the beneficial effects of abciximab (reviewed in [1]).

Drug Monitoring
A number of assays have been developed to monitor GPIIb/IIIa antagonist therapy, and there are positive features and drawbacks to each of the approaches. The author, in conjunction with the scientists at Accumetrics (San Diego, CA), has participated in the development of one of these assays (Ultergra Rapid Platelet Function Assay, Accumetrics, San Diego, CA). It is a fully automated, whole blood, point-of-care test that measures the agglutination of fibrinogen-coated beads by platelets activated by a thrombin receptor-activating peptide. Preliminary results with this instrument indicate that the vast majority (but not all) of the patients receiving abciximab achieve high grade receptor blockade soon after the bolus dose, but there is greater variation in the response during and after the end of the infusion. Moreover, it appears that patients who have less than 70% inhibition of their platelet function as judged by this assay 8 hours after starting abciximab have an increased risk of having a major adverse coronary event after PCI. It remains to be tested,
however, whether abciximab dose adjustment based on this assay, or any other, will result in improved clinical outcomes.

Conclusions
Abciximab has demonstrated efficacy in decreasing both short-term ischemic complications and long-term mortality in patients undergoing PCI. Its role in treating unstable angina, however, is uncertain and may depend on whether the patient undergoes PCI as part of the therapy. Preliminary data are encouraging about its potential role in treating myocardial infarction in conjunction with thrombolytic agents and treating stroke, but definitive studies have not yet been completed. Severe thrombocytopenia is a rare but serious complication, whose impact can be mitigated by ensuring that a platelet count is obtained 2–4 hours after drug administration. Major hemorrhagic complications are also rare but may also be serious when they occur. Platelet transfusions should be considered as treatment for both thrombocytopenia and hemorrhagic complications.

REFERENCES

Introduction


III. Therapeutic Results with Abciximab


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