SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion for the approval of Zenapax. This scientific discussion has been updated until 1 November 2003. For information on changes after this date please refer to module 8B

1. Introduction

Zenapax (INN: daclizumab) is a new active substance intended for immunosuppressive therapy.

The approved therapeutic indication is "prophylaxis of acute organ rejection in *de novo* allogeneic renal transplantation. It is used concomitantly with an immunosuppressive regimen including cyclosporine and corticosteroids in patients who are not highly immunised".

This indication, initially approved for adult patients, has been then extended to paediatric patients through a type II variation.

Daclizumab, the active ingredient in Zenapax, is a recombinant monoclonal antibody expressed in the NSO myeloma cell line. The antibody is directed against the high affinity interleukin-2 (IL-2R) receptor, which is an important mediator of lymphocyte activation, and consequently of graft rejection.

The high-affinity IL-2 receptor is composed of three noncovalently bound chains, a 55-kD alpha chain (also referred to as CD25 or Tac), a 75-kD beta chain, and a 64-kD gamma chain. It is present on all alloactivated T cells, but usually not on resting lymphoid cells. Interaction of IL-2 with this receptor is required for the clonal expansion and continued viability of activated T-cells. By blocking IL-2R daclizumab inhibits proliferation of lymphoid cells, lymphocyte activation is suppressed, and graft rejection is less likely.

The recommended dose for Zenapax in adult and paediatric patients is 1 mg/kg. The medicinal product should initially be given within 24 hours before transplantation. The next and each subsequent dose should be given at intervals of fourteen days for a total of five doses.

2. Chemical, pharmaceutical, and biological aspects

Since the marketing authorisation was granted, the overview under section part II, only the chapter "Stability" has been revised. As the quality variations submitted since the marketing authorisation was granted had no major impact on the safety/efficacy of Zenapax, the quality scientific discussion below reflects, with the exception of the shelf life, the data submitted in support of the initial marketing authorisation. See module "Steps taken after granting the Marketing Authorisation" for information on quality variations in a format of a line listing.

Zenapax is presented as concentrate for solution for infusion. The volume of Zenapax containing the appropriate dose is added to 50 ml of sterile 0.9% saline solution in order to be administered intravenously.

Daclizumab is engineered in a murine myeloma cell line expressing heavy and light chain genes. Material for use in the clinical trials was prepared from cell line SP2/0 using a CMV enhancer promoter system. Due to limited production yields, the antibody was re-expressed in a murine GS-NSO myeloma cell line. The main issue identified during the evaluation of the Part II of the dossier was the establishment of the equivalence between the products derived from these two cell lines.

Extensive biochemical studies indicate that the product derived from the production cell line has similar activity to the original molecule, but with some differences, notably in the amount of incompletely processed N terminus (3% to 40%) and the presence of the 135KD band in the GS-NSO product. Bioequivalence studies in healthy volunteers were conducted to establish the identity of the SP2/0 and GS-NSO materials (see clinical details Part IV).

Composition

One vial with a withdrawable volume of 5 ml concentrate for infusion contains:

25 mg daclizumab, polysorbate 80, sodium chloride, sodium phosphate monobasic, sodium phosphate dibasic, hydrochloric acid, sodium hydroxide, water for injection.

The formulation used in the clinical studies was identical to the proposed market formulation.

The container is a type I flint glass vial (Ph. Eur.) with butyl rubber stopper (Ph. Eur.).

Development pharmaceutics

Studies were conducted for justification of the pH, concentration of stabiliser, buffer type and ionic strength used for formulation of the product. Photolytic stability studies were also performed.

Both of the stoppers used during development are found compatible with the product. Compatibility with the devices recommended for administration was investigated.

Method of preparation

The manufacturing formula for a batch of 20 l is given. The size of a production batch may range from 20 to 200 l. The preparation of the formulated bulk solution is performed in nine steps, starting with the manufacture of the PBS buffer. Part of the PBS buffer is then added to polysorbate 80 and mixed. Bulk substance in PBS buffer is added to a separate vessel in amounts equivalent to the theoretical amounts of protein. The bulk solution is weight adjusted with PBS buffer to approximately 85 % of the final batch volume and mixed. The polysorbate solution is added to the main batch. After mixing, the pH is verified to be 6.9 + 0.1. A final weight adjustment with PBS buffer is performed. The final solution is additionally mixed and subjected to filtration through a sterile 0.2 μm membrane filter into a sterilised receiver vessel. The filtered formulated bulk solution is transferred from the receiver vessel to liquid filler via a presterilised 0.2 μm membrane filter. 5.4 ml of the sterile filtered formulated bulk is dispensed into sterilised and depyrogenated vials. Prior to stoppering of vials, each vial is passed through the nitrogen manifold with filtered nitrogen. Sealed vials undergo inspection performed by visual or automatic control. An extended in-process testing was performed during production of up to three batches in the production scale. Under the conditions used, acceptable product homogeneity was obtained and only minor protein loss was seen during filtration and filling pump purge. The percent antibody monomer remained constant throughout the process.

Steam sterilisation is used for sterilisable machine parts, filter housing with membrane filter, surge systems, rubber components including stoppers and receiver vessels made of glass, glass-lined, or stainless steel. Vials are subject to dry-heat sterilisation and depyrogenation at 350º C for ≥ 10 minutes. The container/closure integrity has been satisfactorily validated. Media fills for validation of the aseptic processing are performed in accordance with GMP.

Control of starting materials

Specifications and routine tests

The specifications for release cover all tests commonly applied to purify protein solutions.

The physical tests for clarity, pH and osmolality represent simple consistency controls of the last step of the purification procedure.

The identity is assured by several methods: peptide map, isoelectric focusing, SDS PAGE under reducing and non-reducing conditions with silver staining, Western blotting, size exclusion chromatography, N-terminal sequence analysis, IL-2 receptor binding assay and the bioassay.

At the same time the above-mentioned methods give evidence of the high purity of the product regarding related proteins, as well as clipped or aggregated molecules. Tests for other impurities, which originate from the cell culture and purification process such as host cell DNA and proteins, are performed.

The test for bioburden requires that the purified bulk solution is close to sterility and the limit for bacterial endotoxins of max. 2.5 EU/mg represents an appropriate safety margin in relation to the generally tolerated amount of max. 5 EU administered intravenously per kg and hour. In addition tests on mycoplasma and virus are performed as in-process controls.

Protein concentration is determined by UV measurement.

The IL-2 receptor binding assay measures the concentration of daclizumab-IL-2 receptor complex by ELISA using horseradish peroxidase labelled goat anti-human IgG as the labelling antibody.

In the bioassay the overall function of the antibody is tested, i.e. the inhibition of activated T cell proliferation by daclizumab in the presence of IL-2. The cell line used in the assay requires IL-2 for cellular proliferation. Daclizumab also binds to the IL-2 receptors of the cells and therefore serves as a competitive inhibitor for IL-2. Inhibition of DNA synthesis, used as a measure of cell proliferation, is determined by 3H-thymidine uptake.

Development genetics

An 8-week female BALB/c mouse was immunised i.v. with human T cells derived from peripheral blood T-cells from a patient with mycosis fungoides. Fusion of spleen cells isolated from the immunised BALB/c mouse to the NS-1 mouse myeloma cell line followed by isolation of hybridoma cells was performed using conventional techniques. From among the positive clones, a single clone was picked and propagated in murine ascites.

The ascites derived mouse monoclonal antibody has been extensively characterised. It was shown to be of the IgG2a subclass. It specifically binds to the 55-kDa subunit of the IL-2 receptor and is reactive with activated human T cells or allogeneic cells. To reduce the risk for an immune response against the murine sequences, the monoclonal antibody was transformed into a chimeric molecule. A humanised antibody was constructed by combining the complementary-determining regions (CDRs) of the mouse monoclonal with human framework and constant regions. To maximise homology, a computer model of the mouse antibody was used and amino acids outside the CDRs, which are likely to interact with the CDRs or the antigen, were retained in the humanised antibody. The variable regions of the heavy and light chains were constructed entirely synthetically and cloned in the appropriate vector construct. After transfection and establishment of a stable SP2/0 cell line producing the "humanised" anti-Tac antibody, the nucleotide sequence of integrated DNA for both the light and DNA sequencing of the corresponding cDNA verified the heavy chain sequence. The determined sequences agreed completely with the expected DNA sequences.

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Cell bank system

The Master Cell Bank (MCB) was established from one frozen ampoule of the pre-seed stock (PSS). A single vial of the MCB was used to prepare the 200 ampoules of the Working Cell Bank (WCB). The current WCB is expected to last 10 years. New WCBs will be prepared according to an approved Standard Operating Procedure.

Fermentation and harvesting

The fermentation process is started by the thaw of one out of three WCB vials and inoculum in a tissue culture flask. Cells are expanded in progressively larger spinner flasks until reaching the size of 1 l. At this stage, cells may be continuously cultured back into a 1l spinner for a maximum of 80 days. Three to six 1l spinner flasks are pooled and used as inoculum for the 30 l bioreactor. The cell culture is successively scaled up from the 30 l via a 80 l and a 400 l bioreactor before seeded into the 2000 l bioreactor used for production. In-process control tests are applied at different steps to monitor glucose, lactate, osmolarity, mycoplasma, bioburden, virus contamination and daclizumab titer.

Purification

The different steps of the purification procedure are: Concentrated broth → Q-Sepharose Chromatography (flow-through collected) →S-Sepharose Chromatography → pH treatment for viral inactivation (pH 3.6 - 3.8, 30 to 35 minutes) →Concentration/Diafiltration → DV50 Filtration for virus removal → Q-Sepharose II Chromatography (flow-through collected) →- Viresolve Filtration for virus removal →Concentration by ultrafiltration → S-300 Gel Filtration Chromatography → Concentration by ultrafiltration → Aseptic filling.

All steps except aseptic filling are performed at 2º - 8º C. The fill is performed at ambient temperature. Details are provided on the equipment as well as on the operating conditions including bed volumes, loading, washing and elution conditions flow rates, regeneration, rinsing and storage of the columns and membranes.

Characterisation

Daclizumab is composed of two identical heavy chain sub-units and two identical light chain subunits linked by disulfide bridges. MS techniques were used for determination of the primary structure and for studies of the heterogeneity of the humanised antibody. Analysis of the amino acid sequence revealed a partial removal of the C-terminal lysine in the heavy chain. In the analysis, no signal could be obtained from the heavy chain N-terminal peptide. This could later be confirmed to be due to the conversion of the glutamine residue to pyroglutamic acid. No modification of the light chain was detected. A single glycosylation site was identified in the heavy chain at Asp296.

Analytical Development

Validation reports for most routine as well as non-routine analyses are provided. The methods used for determination of the *osmolarity, protein content, identity by tryptic map*, as well as contamination with *BSA* and *DNA* are satisfactorily validated. Analysis of pI by isoelectric focusing is used to test for identity. The method used for determination of the *N-terminal amino acid sequence* is satisfactorily validated for specificity and sensitivity. The *HPLC-SEC* method applied for determination of the *monomer form* of the antibody is well validated. The *ELISA* used for *quantification* of daclizumab is satisfactorily validated for accuracy, linearity, reproducibility and limit of quantification. The cell-based *bioassay* used to measure the antiproliferative effect of daclizumab shows rather high intra- and inter-assay variability.

Process validation

Data for validation of the consistency of the current process were collected from five production batches via in-process and release testing. Analytical evaluation of three lots has been completed. Results from in-process control testing during fermentation are only shown graphically. Growth characteristics for all batches appear to be fairly consistent. Analytical evaluation for this batch has not been completed and, thus, it is impossible to conclude whether the deviations seen are acceptable. Titres of 10^8 FFU/ml for retrovirus-like particles are seen in the pre-harvest medium. Removal/inactivation of virus is addressed in section IIV. The consistency of the purification process was studied by analysis of daclizumab yield and purity, endotoxin content, bioburden as well as contamination with DNA and BSA in the different steps.

Control tests on the finished product

The specifications for release are the following:

The physical tests for clarity, colour, pH and osmolality represent simple consistency controls of the composition of the product. Both visible and sub-visible particles are tested.

The identity is assured by isoelectric focusing and the activity measured in the bioassay. The purity with regard to aggregation and fragmentation is routinely controlled by SE-HPLC and SDS PAGE analyses.

The protein concentration is measured by UV determination.

Potency is controlled by the bioassay described for the bulk drug solution. Because the principle of the IL-2 receptor-binding assay is also part of the bioassay the IL-2 receptor-binding assay is not part of the routine release analysis.

In addition bacterial endotoxins and sterility are tested.

Stability

The specifications for the active substance stored under the recommended conditions are the same as for batch release. In addition to the tests and specifications fixed for release analysis, the IL-2 receptor-binding assay is performed in stability studies for finished product.

The proposed shelf life of 18 months is considered acceptable.

The shelf life was extended to 24 months in July 2000 following submission of additional data through a variation to the Marketing Authorisation.

Virus validation

Viral validation studies are performed in accordance with the appropriate ICH guidelines.

Since the cell line used for production contains endogenous retroviruses (X-MuLV), it is important that the production process efficiently and reproducibly removes or inactivates these viruses. The steps that contribute to viral clearance and inactivation are S- Sepharose, Q-Sepharose II, low pH inactivation, DV-50 filtration and Viresolve filtration. Viral clearance studies were performed in order to study the effect of different processing steps to the virus elimination. Down - scaling data is also presented.

The overall viral safety of Zenapax has been demonstrated.

3. Toxico-pharmacological aspects

Zenapax (daclizumab) is a monoclonal antibody that specifically binds to the alpha sub-unit of the IL-2 receptor (IL-2R) complex (also called Tac) and inhibits the binding and biological activity of IL-2 on activated lymphocytes. Interleukin-2 plays an important role in allotransplant rejection thus daclizumab is likely to be effective for the prophylaxis of acute transplant rejection in patients receiving allogeneic renal transplants. It should be administered in combination with an immunosuppressive regimen including cyclosporine and corticosteroids.

The purpose of constructing a recombinant anti-Tac immunoglobin of the human IgG1 isotype derived from a murine anti-Tac IgG2a monoclonal antibody (humanised antibody) is to reduce the immunogenicity of the antibody and improve its pharmacokinetic properties compared with a murine antibody. From pharmacological and toxicological point of view, the primate seems to be the only appropriate species to assess efficacy and safety studies of daclizumab.

Daclizumab used for the pre-clinical/clinical studies was prepared from cell line SP2/0 using a CMV enhancer system whereas the product intended to be marketed is derived from GS-NSO myeloma cell line. The equivalence between these two formulations with respect to binding to IL-2Rα and also to pharmacokinetics after a single dose has been demonstrated.

Pharmacodynamics

In vitro the IC_{50} of 125I-IL2 lymphoblast binding was 3.3×10^{-9} M; and the maximum inhibition of IL-2 stimulated lymphocyte proliferation was 51%. There was little or no uptake of murine anti-Tac by human cells. DAC unlike murine derived anti-Tac, had positive effects on antibody dependent cell mediated cytotoxicity?

In vivo studies of cardiac allograft transplantation in cynomolgus monkeys demonstrated statistically significant advantage against placebo and against murine anti-IL2R. A study of renal allograft transplantation in the cynomolgus monkey gave equivocal results against placebo, possibly due to experimental design and technical problems. In animal models of inflammation positive effects of DAC were shown on autoimmune uveoretinitis and collagen induced arthritis.

No studies have been conducted to explore the secondary pharmacodynamics of DAC other than its immunogenic potential; several experiments demonstrate that anti-Tac antibodies of murine origin are more immunogenic in primates than 'humanised' antibodies.

Pharmacokinetics

Pharmacokinetic studies were performed in both rats and primates, which were also used in the toxicological evaluation of daclizumab. Representative results from pharmacokinetic studies in the monkey are tabulated below, with results from human pharmacokinetic studies for comparison.

Cynomolgus monkey	AUC hr.µg/ml	T½ hr	Cl ml/kg/hr	Vdss ml/kg
1.5 mg/kg	7005	166	0.26	60.8
Expected values in man based on data from renal transplant patients receiving five 1.0 mg/kg doses				
80 kg M aged 45	5298	480	15.1	2.5/3.4*
50 kg F aged 25	6504	409	7.7	1.5/1.9*

*central/peripheral volume

In the cynomolgus monkey male animals had an AUC of 1.5 - 2.2 times that of females, this is likely to be due to the greater clearance in females, amounting to approximately twice that of males.

In the male rat tissue distribution to heart, kidney, liver, and lung was low, with an apparent Volume of Distribution of 130 ml/kg. ^{125}[I]-DAC uptake by the thyroid was 46-48 fold that of the simultaneous plasma concentration at 96-192 hours. Knowledge of metabolism is limited to single dose administration in three male rats. As with metabolism knowledge of the route of elimination of DAC is very limited and seems to rest on the single dose rat study in which 29% of radiolabel was eliminated in urine and 3% in faeces.

Toxicology

Single dose toxicity

Single dose studies in mice and rabbits disclosed no toxic effects. In a toxicokinetic study to GLP standards cynomolgus monkeys received 0, 1.5, 5.0, 15.0 mg/Kg of daclizumab for 28 days. Three animals died, one from anaesthesia/cannula implantation, and two from pulmonary inflammation, possibly due to pneumonia. There were no consistent abnormal physical findings; laboratory abnormalities found were reductions in platelet and leukocyte counts and an increase in serum glucose in males.

Repeated dose toxicity

A repeat dose toxicity study was performed in a clinically relevant cynomolgus monkey model (3/sex/dose). Daclizumab was administered by the clinical route (i.v.) at doses of 1.5, 5.0 or 15 mg/kg/day for 28 days. Control monkeys received daclizumab-vehicle only. Daclizumab displayed a toxicological profile similar to that, which might be expected of an immunoglobulin.

Reproduction studies

No reproductive toxicity studies have been carried out. Given that daclizumab has a very long half-life, that there is no knowledge of similar compounds and that there is a six month 'risk' period post transplantation, it is stated in the Summary of Product Characteristics that women of childbearing potential should use contraception to prevent the risk of pregnancy and continue its use for an additional 4 months after the last dose of Zenapax.

Genotoxicity

No increase in the mutant frequency was observed in the Ames test for any of the tested strains after treatment of 11.4 to 1140 μg/plate of daclizumab. Daclizumab at concentrations of 496-1984 μl/ml did not induce structural chromosome aberrations either in the presence or the absence of metabolic activation.

Carcinogenicity

The applicant has submitted no studies with respect to the carcinogenic potential. The lack of such studies is accepted because of the limited duration of the clinical treatment period and the immunogenic properties of the drug, making conventional carcinogenicity studies inappropriate.

Local tolerance

A venous irritation in rabbits was presented. In this study, daclizumab was well tolerated.

Immunotoxicity

The immunogenicity of daclizumab was evaluated both *in vitro* and *in vivo*. For the in vitro evaluation a immunisation culture system with human peripheral blood mononuclear cells (PBMCs) was used. There was no significant difference in the in vitro antibody formation to daclizumab compared to the negative control. The *in vivo* immunogenicity of daclizumab was examined in monkeys. Two of six monkeys in the intermediate dose group displayed anti-daclizumab antibodies at the end of a 28 days study at concentrations of 260 and 1504 ng/ml, respectively. Furthermore, in a single dose pharmacokinetic monkey study, administration of 1.5 or 15 mg/kg daclizumab did not stimulate antibody development. In conclusion, in these studies it has been demonstrated that the "humanisation process" of the murine antibody was effective in producing an immunoglobulin less immunogenic in primates than the original murine protein.

4. Clinical aspects

The importance of IL-2 in mediating allograft rejection is undisputed, and the benefit of blocking this pathway has been documented in the literature. Monoclonal antibodies directed against the alpha chain (IL-2Rα), which is the most restricted and selective of the three IL-2 receptor chains on activated lymphocytes, have been used in animals and humans. Success was limited by the immune response of the host to the mouse protein, which resulted in accelerated clearance of the administered substance and loss of efficacy.

Daclizumab was constructed as a recombinant humanised monoclonal antibody, containing 90% human IgG1 sequences and 10% murine anti-Tac immunoglobulin sequences, the latter constituting the complementarity determining region of the antibody. The expectation for such a humanised monoclonal antibody would be improved pharmacokinetic properties, as well as decreased immunogenicity, compared with the original murine antibody.

The recommended dose for daclizumab is 1 mg/kg administered intravenously (i.v.) over a 15-minute period. The initial dose should be given within a 24-hour period before transplantation and then every 14 days thereafter for a total of five doses.

The initial SPC, issued at the time of the marketing authorisation, referred to an ongoing study in children and indicated that very limited pharmacokinetic data were available. The study in question was completed and the results were submitted in support of an extension of the indication to children aged five to seventeen years.

Pharmacokinetics

The pharmacokinetic data initially presented were derived from three trials conducted in the target renal transplant adult patient population (Protocols NO14392, NO14874 and NO14393), using a sparse sampling strategy and subsequent population based analysis by nonlinear mixed effect modelling. A population analysis of daclizumab in bone marrow transplant patients was also included in the submission (analysis of Protocols N3681 and NO14348).

The **paediatric Study NO15318** was a Phase I/II open pharmacokinetic and safety study conducted at seven centres in the US. Patients who were less than eighteen years old and receiving renal transplants from living related donors were eligible for inclusion. It was planned to recruit twenty patients from three age groups; five years old or younger, aged six to twelve, and aged thirteen to seventeen years. Patients who had previously been exposed to an IL-2 directed monoclonal antibody were excluded, as were those with lymphocytotoxic cross matches and those with other significant disease or infection. Daclizumab was given as add-on therapy to patients who were receiving 'conventional' immunosuppressant regimens, however these were not specified or standardised.
Daclizumab was given every fourteen days for a total of five doses starting immediately before transplantation.

Following the PK/PD phase of the study, patients were followed for one year to collect safety and efficacy data. The study was closed when the last patient had completed the extension phase.

Table 1. Demographic characteristics of patients in the trial.

Age category		5 and under	5-12	13-17
No. of patients	m/f	10/8	12/6	15/10
Age in years:	mean (sem)	3 (0.4)	9 (0.4)	15 (0.3)
Weight in kilograms:	mean (sem)	12 (0.7)	29 (2.3)	52 (2.9)
Cold ischemia time in hour:	mean (sem)	5 (2.4)	9 (2.6)	15 (2.6)
Donor	living/cadaveric	15/3	10/8	16/9
No. receiving five doses		14	17	22

Population pharmacokinetic methods were used to derive pharmacokinetic indices for an average paediatric patient. The results, with historical adult values, are shown in Table 2. The inter-individual variability in children is said to compare favorably with that in adults. However, the various comparators suggest a variability of up to twice that in adults.

Table 2. PK indices for adults and children

	Clearance (l/h)	V1 (l)	V2 (l)	t1/2 (h)
Children	0.01	1.94	1.36	313
Adults	0.15	2.49	3.43	480

V1 =vascular; V2 = extra-vascular

On request of the CPMP, individual data were provided, together with details of the methods used to derive the pharmacokinetic indices. Daclizumab levels were measured in 542 blood samples from 124 patients. The data were analysed using the NONMEM (non-linear mixed effect model) computer programme, which uses an iterative process to derive 'best fit' variables for the data. The resulting model is then tested for predictive function against observed data from some patients, which have been reserved prior to the model development. Similar methods were used for the paediatric study. The factors found to influence plasma clearance were increasing body weight, and age, black patients and those with proteinuria were found to have lower plasma clearance.

Pharmacodynamics

By using fluorescence-labelled antibodies that recognise different epitopes of the receptor complex and fluorescence-activated cell sorter (FACS) analysis, it was determined that daclizumab bound to the alpha chain (Tac) of the IL-2 receptor on peripheral blood lymphocytes (PBL) and that these cells are not eliminated from the circulation, nor loose the Tac receptor from the cell surface.

Adults

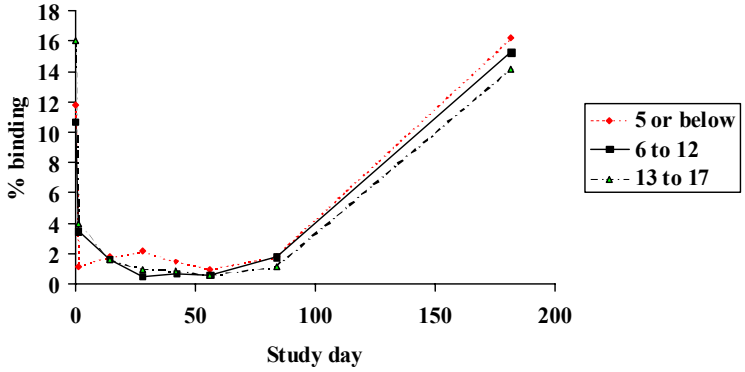
Studies were performed in renal transplant patients who received multiple doses of daclizumab, either 0.5 or 1.0 mg/kg once a week or once every other week for a total of five doses in a phase I study (Protocol NO14392), or 1.0 mg/kg once every other week for a total of five doses in the triple-therapy phase III study (Protocol NO14393) in which 10 daclizumab-treated patients were compared with 10 placebo-treated patients. The Tac receptor remained saturated for as long as 2 months after the last dose of daclizumab. The return to baseline levels of CD25+ cells occurs after four months.

Children

The uptake of the antibody 2A3, which recognises CD25 exhibited on activated T-Cells, was measured at various time points. Uptake of the monoclonal 7G7/B6 (which is not blocked by daclizumab) served to measure non-specific changes. Group mean changes in 2A3 binding are shown in Figure 1.

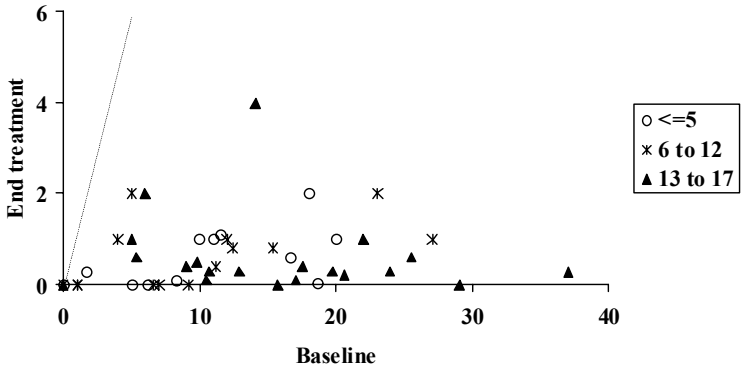
Minor reductions and quite a different pattern with time were seen for 7G7/B6.

Fig. 1 Plasma binding of 2A3 antibody by age group and study day. Daclizumab treatment ended on day 56.



An idea of the scatter of individual and age group responses can be gained from Figure 2, which shows the CD25 expression before and at the end of five treatments with daclizumab, i.e. the expected nadir. The reduction in expression is generally impressive and no age related differences are evident.

Fig. 2 Percentage expression of CD25 on peripheral blood mononuclear cells, by patient and age group, at baseline (taken as Day –1 or 0) and end of treatment ~ Day 56. Dotted line is line of identity.



Anti-daclizumab antibodies

Twenty of 58 (34%) evaluable patients were considered to be positive for anti-daclizumab antibodies. Although no temporal relationship was observed between the onset of acute rejection episodes and the measurement of positive antibody levels, the low rate of acute rejections during the study (17%) limited the ability to assess this relationship.

A significantly higher rate of anti-daclizumab antibodies in children (~ 34%) compared to the rates in the adult studies (~ 9%) provided in support of the marketing authorisation holder application was observed. While this might be related to different patterns of steroid use no facts are advanced to

support this hypothesis. The antibody development has not been shown to have any clinically relevant effects.

Efficacy

Adults

Dose selection

All patients were to receive a total of five doses of 1.0 mg/kg of daclizumab or placebo administered as a 15-minute intravenous infusion. The first dose was given immediately before transplantation.

Subsequent doses were given every 14 days, and patients were to complete the course of treatment even if rejection occurred. 80-83% of randomised patients received all five doses. The dosage regimen was based on the following considerations:

- In trial NO 14392, no rejection episodes were observed at a dose of 1 mg/kg
- Pharmacokinetic modelling using data from renal transplant patients showed that a dose of 1 mg/kg given every 2 weeks would maintain serum trough concentrations above 5 mg/ml for the first 70 days post-transplant.
- In vitro IL-2 receptor binding and IL-2-dependent lymphoblast proliferation assays indicated that a concentration of 5 to 10 mg/ml was sufficient to saturate the alpha chain of the IL-2 receptor and eliminate IL-2 stimulated proliferation of T-lymphocytes.
- No dose-limiting toxicity was observed either pre-clinically or clinically in the phase I studies.

Efficacy endpoints

In Study No.14393 daclizumab was added to background immunosuppression with cyclosporin, prednisolone, azathioprine.
In Study No.14874 daclizumab was added to background immunosuppression with cyclosporin and prednisolone.
For both phase III studies, the primary efficacy endpoint was the proportion of patients experiencing biopsy-proven acute rejection in the first 6 months post-transplant. This endpoint has been frequently used in transplantation studies and is generally accepted, although, its validity as a surrogate marker for long-term graft survival may be incomplete. The incidence of biopsy-proven rejection at 12 months and three years was evaluated secondarily. Other secondary efficacy endpoints included:

- Time to biopsy-proven acute rejection
- Number of acute rejection episodes per patient
- Graft survival at six months, 12 months and three years
- Patient survival at six months, 12 months and three years
- Cumulative dose of corticosteroids and use of antilymphocyte therapy for rejection
- Incidence of delayed graft function
- Graft function at six months
- CMV infections during the first six months

Acute rejection

Daclizumab reduced the incidence of biopsy proven-acute rejection in the first 6 months post-transplant from 47% to 28% in NO14874 (double therapy) and from 35% to 22% in NO14393 (triple therapy) (Table 3).

Table 3. Biopsy-Proven Acute Rejection at 6 Months Post-transplant

	Study NO14874		**Study NO14393**	
	Placebo (N=134)	Daclizumab (N=141)	Placebo (N=134)	Daclizumab (N=126)
Number of patients with acute rejection	63 (47%)	39 (28%)	47 (35%)	28 (22%)
P value*	0.001		0.03	

*Cochran-Mantel-Haenszel test stratified by centre

The time to biopsy-proven acute rejection was significantly longer in daclizumab-treated patients than in placebo-treated patients in both studies.

Graft survival at 6 months post-transplant was numerically improved in the daclizumab group compared with the placebo group in both trials, and the difference was statistically significant in the triple-therapy study (Table 4). The most common cause of graft loss in the first 6 months post-transplant in the daclizumab group was technical surgical complications (7 of 13 daclizumab patients in NO14874 and 2 of 3 daclizumab patients in NO14393). The most common cause of graft loss in the placebo group was death with a functioning graft (5 of 19 placebo patients in NO14874 and 4 of 12 placebo patients in NO14393).

Table 4. Graft Survival at 6 Months and 1 Year Post-transplant

	Study NO14874		Study NO14393	
	Placebo (N = 134)	**Daclizumab (N = 141)**	**Placebo (N = 134)**	**Daclizumab (N = 126)**
6 Months Post-transplant				
No. of patients with functioning graft	115 (86%)	128 (91%)	122 (91%)	123 (98%)
P value	**0.22***		**0.023[**	
1 Year Post-transplant				
No. of patients with functioning graft	111 (83%)	124 (88%)	121 (90%)	120 (95%)
P value	**0.300***		**0.078***	

*Stratified logrank test, [Unstratified logrank test.

A trend to improvement in graft survival in daclizumab patients compared with placebo patients was maintained at one year post-transplant. Numerically more graft losses from acute or chronic rejection were seen in the placebo group than in the daclizumab group in both studies. Graft loss was defined as return to chronic dialysis, transplant nephrectomy, retransplantation, or death. Technical complications were mainly renal artery and/or vein thrombosis.

Patient survival at 6 months post-transplant was numerically better in patients treated with daclizumab, compared with those treated with placebo in both studies; the difference was statistically significant in the double-therapy study (Table 5). No daclizumab-treated patients died of infection in the first six months post-transplant, while seven placebo patients died as a result of infection in the first six months.

Table 5. Patient Survival at 6 Months and 1-Year Post-transplant

	Study NO14874		Study NO14393	
	Placebo (N = 134)	**Daclizumab (N = 141)**	**Placebo (N = 134)**	**Daclizumab (N = 126)**
6 Months Post-transplant				
No. of patients alive	128 (95.5%)	141 (100%)	130 (97.0%)	125 (99.2%)
P value*	**0.01**		**0.19**	
1 Year Post-transplant				
No. of patients alive	126 (94%)	140 (99%)	129 (96%)	123 (98%)
P value*	**0.013**		**0.512**	

The trend to improvement in patient survival in daclizumab patients was maintained at one year post-transplant in both studies, and the difference between the two treatment arms in the double-therapy study remained statistically significant (p = 0.013). A total of four daclizumab patients and 13 placebo patients had died by 1 year post-transplant. Causes of death are dealt within the safety section.

Additional Secondary Analyses

The cumulative dose of corticosteroids during the first 6 months post-transplant was lower in the daclizumab group than in the placebo group in both studies (3132 vs. 3622 mg in NO14874 and 4314 vs.

4184 mg in NO14393), but the difference was statistically significant only in the double-therapy study (p = 0.01). A smaller proportion of patients in the daclizumab group required additional antilymphocyte therapy to treat rejection than in the placebo group in both studies. The difference was statistically significant in the double-therapy trial (8% vs. 16%, p = 0.02). Graft function at 6 months post-transplant was significantly better in the daclizumab group than in the placebo group in the double-therapy study and equivalent in the daclizumab and placebo groups in the triple-therapy study. At 1 year post-transplant, no difference was seen in renal function between the daclizumab group and placebo group in either study with the exception of serum creatinine levels, which were significantly lower in the daclizumab group than in the placebo group in the double-therapy study. The occurrence of CMV infections was used as a marker for detecting the potential for overimmunosuppression. The incidence of CMV infections was lower in the triple-therapy study than in the double-therapy study, correlating with the requirement in the triple-therapy protocol for CMV prophylaxis.

Long term patient survival and graft survival rates

Table 6. Principal outcomes of the Phase III studies at three years post transplant

Study NO14393 (+ triple immunosuppression)		
	Placebo n = 134	**Daclizumab n = 126**
Patient survival	126 (94.0%)	116 (92.0%)
Graft survival	111 (83%)	106 (84%)
Median and range GFR (ml/min)	43 (15 – 117)	48 (12-91)
Biopsy proven chronic rejection*	8 of 11	12 of 19
Lymphoproliferative disorders or other neoplasm	9 (6.7%)	10 (7.9%)
Study NO14874 (+ double immunosuppression)		
	Placebo n = 134	**Daclizumab n = 141**
Patient survival	118 (88.1%)	135 (95.7%)
Graft survival	105 (78%)	116 (82%)
Median and range GFR (ml/min)	53 (15 – 99)	54 (22 -112)
Biopsy proven chronic rejection*	10 of 22	6 of 16
Lymphoproliferative disorders or other neoplasm	12 (9%)	7 (5%)

*biopsies were optional and therefore infrequent

In Study No.14393, the three-year patient survival rate was 92% for daclizumab and 94% for placebo (table 6); the equivalent graft survival rates were 84% and 83%. Neither difference from placebo is statistically significant.

In Study No.14874, the three year patient survival rate was 96% for daclizumab and 88% for placebo (p = 0.017), showing a statistically significant advantage in patient survival; the equivalent graft survival rates were 82% and 78%, showing a non statistically significant difference between placebo and daclizumab in this triple immunosuppressant trial (table 6).

A pooled analysis of graft survival from both studies (Bumgardner et al., Transplantation 2001 72:839-45) showed a non-significant advantage for graft survival at three years (p = 0.3).
The analysis set out very clearly the one and three year results of the two studies and the pooled outcome. Some patients on triple immunosuppression were titrated downward to double immunosuppression and some on double immunosuppression had the components changed e.g. swapping mycophenolate for azathioprine. It was considered that double or triple immunosuppressant regimens could be regarded as standard in the EU. Therefore, the CPMP considered that the pooled analysis was a reasonable reflection of the long-term "real life" experience of daclizumab. The author of this publication commented that, given the efficacy of daclizumab, the trials were not powered to

show a difference at three years; the authors' power calculation suggests a trial of 1,200 patients per treatment might have been necessary.

Children

Acute rejection
Five out of sixty (8.3%) transplanted patients had a biopsy-proven acute rejection during the first six months after transplantation, and a total of ten (17%) in the first twelve months (one patient had two episodes). An additional patient experienced a presumed acute rejection episode. The acute rejection episodes were evenly distributed among the three age groups. Six rejection episodes were classified on biopsy as mild. Two episodes were classified as moderate; one episode was classified as borderline change and one was classified as acute rejection with no grade specified.

For reference the six-month rejection rates in the two adult Phase III studies were 22% and 28% (it is unclear if this is all rejections or biopsy proven rejections.)

Delayed graft function
Three patients experienced delayed graft function; two had improved renal function by one week and the third by two weeks post-transplant.

Graft Failure
Two patients experienced graft loss; one due to renal vein thrombosis, the other due to focal segmental glomerular sclerosis.

Safety

Adults

The daclizumab safety database contained data on 518 patients at the time of the Marketed Authorisation, who received at least one dose of daclizumab in studies conducted in three different indications: renal transplantation, bone marrow transplantation, and Tac-bearing tumours. The assessment focuses on the pooled data from the four renal transplant studies, which included 336 patients who received daclizumab and 293 patients who received placebo.

More than 80% of the patients in the four renal transplant studies received all five doses of study drug. Patients who received less than five doses of study drug were considered to have not completed the study and were classified as prematurely withdrawn. The rates of premature withdrawal in the daclizumab group and the placebo group were similar.

The number of daclizumab-treated patients who died during the first year post-transplant was significantly less than the number of placebo patients who died (5 of 336 daclizumab patients vs. 13 of 293 placebo patients, $p = 0.03$). All but one death occurred in the pivotal trials. None of the deaths in the daclizumab groups were assessed as related to study drug. Infection was the most common cause of death in the placebo groups and was responsible for 6 of the 13 fatalities. The addition of daclizumab to double or triple therapy did not increase the overall incidence of serious adverse events or change the type of serious adverse events reported. Infections were the most frequent serious adverse events considered possibly or probably related to study drug and were reported in 2.4% of the patients in the daclizumab group and 4.1% of the patients in the placebo group.

Table 7. Overview of Serious Adverse Events

	Placebo (N = 293)	Daclizumab (N = 336)
All Serious Adverse Events (Including Unrelated)		
Total No. of patients with one or more serious adverse events	130 (44.4%)	134 (39.9%)
Total No. of serious adverse events	237	240
Possibly or Probably Related Serious Adverse Events		
Total No. of patients with one or more serious adverse events	22 (7.5%)	14 (4.2%)
Total No. of serious adverse events	28	17

Note: Infections and laboratory abnormalities are included as adverse events.

Infections

Most patients experienced infections, but no difference was seen between daclizumab-treated patients and placebo patients with respect to either the incidence of infections or the types of infection, except for cellulitis and wound infections, which were more frequent with daclizumab. One daclizumab-treated patient died of infection, compared with seven in the placebo groups.

Lymphoma and other malignancies

At one year post-transplant, two daclizumab-treated patients (0.7%) and two placebo patients (0.7%) had developed a lymphoma. Both daclizumab patients were from the triple-therapy study, and one of these patients had received only one dose of drug before being withdrawn from the study. One placebo patient in each study developed lymphoma. At 1 year post-transplant, a total of four daclizumab-treated patients and five placebo patients had developed non-melanoma skin tumours.

Hypersensitivity reactions

Two cases of severe hypersensitivity reactions following administration of Zenapax have been reported.

Long-term safety

Review of the causes of death from the three-year results of studies No14393 (placebo group: n = 134; daclizumab group: n = 126) and No14874 (placebo group: n = 134; daclizumab group: n = 141) showed that in the placebo group three patients died from brain haemorrhage, one from hypoglycaemia, and seven from sepsis/malignancy. In the daclizumab group there were no deaths due to brain haemorrhage and four patients died due to sepsis/malignancy.

Safety of the concomitant use of Zenapax with another antilymphocyte antibody therapy

Study NR15880, an ongoing multi-centre (initiated in August 1999), randomised, double-blind, placebo-controlled trial in patients receiving a first cardiac transplant, assesses the effect on acute graft rejection of the addition of daclizumab to immunosuppressive therapy which includes mycofenolate mofetil (3 g/day), cyclosporin, and steroids. Daclizumab was administered as a 1-mg/kg infusion within twelve hours of transplantation and repeated at 14-day intervals for a total of five doses.

Table 8 below shows the number of patients dying, experiencing graft rejection, and the general classification of cause of death. It is apparent that although less patients experienced acute rejection episodes in the active treatment arm there were more deaths.

Table 8. : Patients dying and experiencing graft rejection at twelve months

	Daclizumab (n= 216)	**Placebo (n=218)**
Mortality at 12 months	21 (9.7%)	12 (5.5%)
Graft rejection at 12 months	97 (44.8%)	116 (53.4%)
Death from infection	10	0
Death from shock and multi-organ failure	2	4
Cardiac death	2	5
Other cause of death	7	3

Nine patients in the daclizumab group and none in the placebo group had an infection that was directly implicated in the cause of death. In addition, there were five other daclizumab patients and four placebo patients who had an active infection at the time of death but in whom the infection did not seem to be directly implicated in the death of the patient.

Risk factors associated with mortality and infection

The daclizumab and placebo patients were well matched for most baseline characteristics and preoperative conditions. However, there was some imbalance with regard to the proportion of diabetic

patients in the two groups: for the daclizumab group 35 (16%) were diabetic; 20 (10%) of placebo patients were diabetic.
The use of maintenance immunosuppression was similar in both groups. The frequency of anti-lymphocyte therapy (OKT3, ATG, ATGAM) use was also similar in both groups. However, eight of forty patients receiving daclizumab and anti-lymphocyte therapy eight died, whereas two of thirty-seven patient receiving anti-lymphocyte therapies died.
Higher cumulative doses of steroids were used to treat rejection during 91 to 180 days and 181 to 365 days in patients receiving Daclizumab compared to that used in the placebo group (Table 9).

The population used to determine the role of infection and study drug in a patient's death was the safety population and not the intent to treat population, i.e. patients who received at least one dose of study drug and who had at least one observation post randomization. Using these numbers and excluding patients who died more than 90 days after receiving the last dose of daclizumab, 10/216 (4.6%) of daclizumab patients died with an infection in which daclizumab could possibly be implicated, compared to 4/207 (1.9%) of patients receiving only placebo.
The imbalance between placebo and daclizumab deaths with respect to concomitant use of antilymphocyte therapy seems more likely to be a chance finding due to small numbers rather than a true interaction.

Children

Ninety-eight percent of patients entered into the study NO15318 received five doses of daclizumab; four patients received six doses. No patients died during the study.

Study withdrawals due to adverse events

Two patients withdrew from the study due to adverse events. A four-year-old girl experienced renal vein thrombosis on the second day after transplantation. A thirteen-year-old boy experienced poor renal function post transplantation due to focal segmental glomerulosclerosis.

Serious adverse events

Serious adverse events resulted in discontinuation of daclizumab in two cases. None of the remaining 49 serious adverse events required adjustment in daclizumab dosage or administration. Of the 51 serious adverse events, 49 (96%) resolved with no sequelae, one resulted in graft loss and one was considered unresolved (asthma).
Review of the nature of the serious adverse events shows that they are highly likely to be related to transplantation surgery and not to treatment with daclizumab. There are no evident differences in the patterns between age groups. However if there were differences, in reality, it is unlikely that they would show up between small groups studied.

Adverse events

Adverse events were experienced by 98.4% of patients (i.e. one patient did not have any adverse events). Given the severity of the underlying disease, the major surgical intervention, the concomitant immunosuppression, the lack of a placebo or an alternate dosage group, and the relatively small size of the study, the CPMP has considered that no meaningful information can be drawn from the analysis, at least in regard to a causative association with daclizumab treatment.

Laboratory abnormalities

The nature and frequency of those experienced, including those related to renal function is as might be expected.

Risk/benefit assessment
Daclizumab fulfilled the requirements of providing enhanced immunosuppressive efficacy without appreciably increasing the risk of complications due to overimmunosuppression. Although only recipients

of cadaveric grafts were included in the Phase III trials, the data from Phase I/II trials and the general mechanism of action of daclizumab support extrapolation of the findings to recipients of living donor kidney allografts. The data demonstrated a good safety profile of Zenapax during the three years follow-up period.

The overall benefit/risk ratio of daclizumab in the indications applied for has been considered as favourable.

The marketing authorisation has been therefore recommended.

5. Overall conclusions and benefit/risk assessment

On the basis of the assessment of the dossier as described in this report, as well as the type II variation submitted to extend the therapeutic indication to include paediatric patients, and the type II variation submitted to include the long term follow-up data from the two pivotal trials in the product information, the CPMP has evaluated in depth the risk/benefit balance and amended the Summary of Product Characteristics in order to ensure that all considerations were properly reflected. A positive opinion was adopted for Zenapax for the following indication in adult and paediatric patients:

"Zenapax is indicated for the prophylaxis of acute organ rejection in *de novo* allogenic renal transplantation and is to be used concomitantly with an immunosuppressive regimen, including cyclosporine and corticosteroids in patients who are not highly immunised".