

SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion and scientific discussion on procedures, which have been finalised before 1 October 2002. For scientific information on procedures after this date please refer to module 8B.

1. Introduction

LeukoScan consists of the Fab' fragment of the antibody Sulesomab : this parenteral monoclonal antibody is produced in murine ascites fluid and is formulated for direct labelling with ^{99m}Tc with binding activity to both carcinoembryonic antigen (CEA) and surface granulocyte non-specific cross-reacting antigen (NCA90) which is present on virtually all neutrophils. As such, it was anticipated to be a good carrier for targeting areas where granulocytes have accumulated and potentially useful for imaging infection and inflammation foci.

LeukoScan is presented as a lyophilised powder to be reconstituted with approximately 1100 MBq of sodium pertechnetate ^{99m}Tc in sterile isotonic saline solution for intravenous administration. The finished medicinal product LeukoScan is presented as a kit consisting of one vial containing the sterile, non-pyrogenic, lyophilised powder, which is to be mixed with ^{99m}Tc solution after reconstitution. ^{99m}Tc is not provided in the kit.

The reconstituted product is indicated for diagnostic imaging for determining the extent and location of inflammation and infection in bone in patients with suspected osteomyelitis. In particular LeukoScan has been developed for the diagnosis of osteomyelitis complicating diabetic foot ulcers or various lesions in other long bones. Osteomyelitis is one type of lesion which can be difficult to diagnose, particularly when an area of intense inflammation is nearby. The detection of occult sites of infection and inflammation is an integral part of the clinical evaluation of the febrile patient. The anatomical delineation of the site and extent of local inflammation is critical to the clinical management of the infectious processes, both for diagnosis and for monitoring the response to therapy.

The anatomical site of the inflammatory processes and whether major structural derangement has occurred at the site determines the efficacy of an imaging technique. The use of a patient's autologous leukocytes for detection is a non-invasive diagnostic technique used in clinical practice. Labelling White Blood Cell Scanning with ^{111}In or ^{99m}Tc hexamethyl propylene amine oxime present major problems such as: requirement for a blood sample from a patient who is frequently critically ill, as well as a potential hazard of infecting nuclear medicine personnel handling leukocytes from high-risk patients. ^{67}Ga scintigraphy is also being used, but it has poor resolution, imposes high radiation dose to the patient, the interval between administration and imaging is long and it is not always available to the nuclear medicine facilities. ^{99m}Tc has advantages over other radionuclides, including ^{123}I and ^{111}In . Its shorter half-life results in lower dose exposure to a patient when the same activity is injected and it is available and convenient to the user.

2. Chemical, pharmaceutical and biological aspects

LeukoScan consists of the Fab' fragment of the antibody Sulesomab : this parenteral monoclonal antibody is produced in murine ascites fluid and is formulated for direct labelling with ^{99m}Tc with binding activity to both carcinoembryonic antigen (CEA) and surface granulocyte non-specific cross-reacting antigen (NCA90) which is present on virtually all neutrophils. As such, it was anticipated to be a good carrier for targeting areas where granulocytes have accumulated and potentially useful for imaging infection and inflammation foci.

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sterile, non-pyrogenic, lyophilised powder, which is to be mixed with ^{99m}Tc solution after reconstitution. ^{99m}Tc is not provided in the kit.

Composition

The active substance of this medicinal product is a mixture of Fab'-SH, (Fab')₂, heavy and light chains and other fragments.

The excipients used are: stannous chloride (as a reducing agent), sodium potassium tartrate and sodium acetate (they prevent the precipitation of stannous chloride at physiological pH by acting as weak chelating agents), sodium chloride and sucrose as protein stabilisers (for example during lyophilisation).

The active substance is produced by ascites technology. Injecting mice produce murine ascites fluid after pristane priming, with the hybridoma cell inoculum. The hybridoma was produced by fusion of lymphocytes from CEA immunised mice and the SP2/0 mouse myeloma cell line. Ascites develop 14 to 28 days following inoculation and the fluid is removed from the mice by a process known as "tapping". The ascites fluid contains antibodies (Sulesomab IgG) produced by the hybridoma cell line, which are shown to bind to granulocytes. Sulesomab IgG is then purified from the ascites fluid and processed by digestion with pepsin to produce Sulesomab F(ab')₂. The final step in the process is the reduction of the Sulesomab F(ab')₂ with cysteine.

The applicant has justified the choice of Sulesomab rather than the intact antibody or divalent F(ab')₂ because of the following:

- i) monovalent Fab' is rapidly cleared from blood to permit imaging within an hour of administration,
- ii) there are free thiol groups on the Fab' following chemical reduction for direct radiolabelling with ^{99m}Tc
- iii) Fab' is less immunogenic and at the dose chosen, no development of human anti-murine antibodies (HAMA) were developed.

The applicant has provided information on the two formulations used in the clinical trials: the differences between these two formulations are not significant in terms of ratio of the active constituent to excipients which remains unchanged. A third formulation intended for marketing has been also developed with a higher amount of stannous ion (see table I).

The initial formulation chosen for SulesomabScan, and used in Phase I, II and early Phase III clinical trials was based on the formulation for another Fab'-SH monoclonal antibody product (formulation 1). However, the Phase II studies showed that Sulesomab, was effective as an imaging agent at doses much lower than those previously used for the other Fab'-SH monoclonal antibody product. Formulations 1 and 2 were used in respectively 66% and 34% of the patients who entered into the pivotal clinical trials.

The commercial formulation was afterwards developed, which contained three times the amount of stannous ion which resulted in satisfactory labelling of the Fab' fragment. Three commercial batches have been shown to be physico-chemically equivalent to formulation 2. Furthermore, an in vivo study has been carried out to demonstrate bioequivalence of the commercial formulation and the clinical trial formulation.

Method of preparation

Murine ascites fluid containing Sulesomab IgG is purified and digested with pepsin to F(ab')₂ and then reduced to Fab'-SH using cysteine. The yield from processing up to 3 l of ascites fluid ranges from 8 000-12 000 vials of lyophilised final product.

The solutions of the excipients used in the final product are prepared and filtered. These solutions are added gradually to the Fab'-SH solution. An in-process control is instituted to check the concentration of Fab'-SH by absorbance at 280 nm before the formulated solution is filtered through 2 filters which are arranged in tandem into vials for lyophilisation. The actual manufacturing formula as well as the details in the form of a flow chart including critical in-process control parameters was provided. The

applicant set also an appropriate prefiltration bioburden limit for the definitive sterilisation step in the manufacturing process.

Control of starting materials

The source material for Sulesomab bulk IgG is ascites fluid, which is produced, in pristane-primed mice (CD2F1) following inoculation of the mice with Sulesomab hybridoma cells. Sulesomab ascites fluid, stored at -20 °C by Charles River Laboratories (CRL) and tested to assure it meets specifications, is thawed at 37 °C (approximately one hour), centrifuged at 15 000 to 20 000 x g and filtered through sterile pyrogen-free-glass wool, by CRL, prior to shipment to Immunomedics. The centrifuged ascites fluid is shipped at 2-8°C, received at Immunomedics and stored at 2-8 °C for a maximum of 48 hours prior to loading onto the Q-Sepharose column.

The ascites fluid harvests are subject to extensive viral testing where they have to be tested negative before they are released.

The mice used in production are monitored according to the CPMP guideline on Production and Control of Monoclonal Antibodies of Murine Origin. CDF-1 and Balb-C strains of mice are used in the production. Reassurance was provided that the CDF-1 and Balb-C mice will be monitored as far as viral safety is concerned in compliance with the relevant CPMP guidelines.

Both the Master Cell Bank (MCB) and the Manufacturer's Working Cell Bank (MWCB) have been tested virologically and bacteriologically according to the Notes to Applicants for Marketing Applications on the Production and Quality Control of Monoclonal Antibodies of Murine Origin Intended for Use in Man. Tests for ecotropic and xenotropic viruses and also an in vitro test for adventitious agents have been carried out. MWCB but not MCB has been tested to contain reverse transcriptase activity.

The down stream process encompasses seven steps; three chromatographic steps for the purification of IgG, the enzymatic cleavage by pepsin to F(ab')₂ and subsequent chromatographic purification and a chemical conversion to the active substance Fab'-SH followed by chromatographic purification. The process is very similar to the strategy adopted for another product manufactured by the company. In order to avoid cross-contamination, the company gave written assurance that the columns are product specific. Details relating to sanitation, equilibration of the columns and elution conditions has been reasonably well documented.

In process specifications have been provided to ensure satisfactory progress of the production. The quality of the intermediate is monitored by IEF, HPLC, immunodiffusion to isotype the IgG, immunoreactivity and SDS-PAGE. Furthermore, tests including amplified XC plaque, MAP, amplified S+L-focus and reverse transcriptase are also carried out to ensure viral safety. Other control tests include DNA content, pristane, bioburden and endotoxin. In addition, in-process controls have been included to monitor the pepsin digestion and cysteine reduction steps.

The specification for Fab'-SH contains four types of control tests to determine

- i) the potency, e.g., an immunoreactivity test; justification for the use of this assay and the proposed control limits are required. During the hearing with the company at the BWP, the company agreed to establish an appropriate potency assay (flow cytometric assay) in addition to the currently used immunoreactivity assay and to provide the full validation report within 3 months
- ii) the purity eg. HPLC, IEF
- iii) uptake of ^{99m}Tc by Fab' radiolabelling (SEC) and free ^{99m}Tc (ITLC)
- iv) bioburden and endotoxin.

Control tests on intermediate products

It was noted that the reference standards prepared for Fab' bulk were manufactured by a process not in compliance with the proposed manufacturing process, e.g. in the case of Fab'-SH a different chemical modification method was chosen. However consistency in the manufacturing process was proven with strict limits on the specifications for each of the intermediates.

Control tests on the finished product

The proposed specification is extensive utilising a battery of physico-chemical tests to control the finished product. The purity of the finished product is tested by 3 different methods; HPLC, IEF and SDS-PAGE.

The major bands on the IEF and SDS-PAGE are quantified by densitometry.

ITLC method is used for the determination of ^{99m}Tc-labelled antibody. Size-exclusion chromatography with Gamma-Ram detector is also employed to determine the ^{99m}Tc incorporation of the product. The company supplied sufficient data on radiolabelling efficiency.

The results of a series of tests addressing the issue of batch-to-batch consistency, particularly with reference to mouse serum albumin, host immunoglobulins and Sulesomab IgG were provided.

The information submitted demonstrated batch-to-batch consistency by:

- reasonable consistency of the yield of intermediates
- strict limits on the specifications for each of the intermediates and finished product
- consistency of molecular homogeneity tested by SDS-PAGE and HPLC,
- carbohydrate analysis of the finished product,
- consistency in secondary and tertiary structure tested by circular dichroism,
- consistency in primary structure tested by C and N terminal amino acid analysis.

Stability

Stability studies have been carried out on the intermediates, the bulk drug substance and the finished product. No storage times have been proposed for other intermediates although stability data have been provided. For the bulk drug (Fab'-SH), the company has provided 11 day stability data to justify their position that the product should be lyophilised within 10 days. Data justify the proposed shelf life of 18 months.

Process validation

A summary of the potential impurities are tested for on starting materials, intermediates and/or finished product, are provided along with the limits of detection in the following table:

Potential Impurity	Test	Limit of Detection
Bovine Spongiform Encephalopathy (from fetal calf serum)	NA*	NA*
Pyrogen++	LAL	0.001 EU/mL
Microorganisms	Sterility	NA
Mycoplasma	Mycoplasma Assay	NA
Murine Retroviruses	XC Plaque, S ⁺ L ⁻	NA
Viruses	MAP, Cocultivation with Vero, MRC-5 and 2.775 cells	NA
Protein A	Protein A Assay	2 ng/mL
Pristane	Pristane by GC	260 ppm+++
Murine DNA	Murine DNA Assay	1 pg/mg
Aggregates	HPLC	1%
Degradation Products	HPLC	1%

NA = Not Applicable, are biological tests

LAL = Limulus Ameboocyte Lysate

MAP = Mouse Antibody Production

GC = Gas Chromatography

HPLC = High Pressure Liquid Chromatography

* = The supplier of the fetal calf serum (Hyclone) certifies that all serum is collected from USDA inspected abattoirs in the USA

The residual levels of pepsin in the finished product for three lots is less than 3 ng per 0.3 mg i.e. 10 ppm. The levels found in the corresponding lots of F(ab')₂ bulk were between 5 and 50 ppm.

Viral safety

Studies have been carried out to validate the process of viral burden reduction. Three model viruses, XMuLV, pseudorabies and SV40 were selected for the validation studies in order to examine viral reduction capacity for four steps of the down stream process:

- Q-sepharose chromatography for ascitic fluid fractionation,
- protein A chromatography, for purification of IgG,
- pepsin digestion procedure
- S-sepharose column for purification of F(ab')₂ after pepsin digestion.

For the single use columns, Q-sepharose and S-sepharose, two unused columns prepared in an identical manner were used for each viral spiking study. Protein A columns were validated for re-use for up to 20 runs where duplicate studies were carried out using a unused column and a reused column, which had been recycled for 20 times.

Substantially the effective mechanism for the reduction of the viral burden by the manufacturing process of LeukoScan is the inactivation of viruses by low pH treatment realised in the pepsin digestion step (37 °C, 90-100 min, pH 3,7) and the following S-Sepharose chromatographic step (4 hours processing time at pH 4.0).

The BWP during its meeting on 7-8 October 1996 considered that there are weaknesses in the capacity of the manufacturing process to remove/inactivate non-enveloped viruses

The effective mechanism for reduction of the viral burden by the manufacturing process of LeukoScan is the inactivation of viruses by low pH treatment during the pepsin digestion step and the S-Sepharose step. Although experimental studies provided did not sufficiently meet the requirements of the CPMP Guidelines, there are some indications that the low pH treatment is effective in inactivating enveloped viruses. However, validation studies do not provide full confidence that non-enveloped viruses are effectively removed.

Therefore, the company should carry out viral testing in accordance with Annex 1 Table 2 of the CPMP Note for Guidance on the Production and Quality Control of Monoclonal Antibodies on a routine basis. No detectable viruses should be present in the ascitic fluids except for retroviruses. No viruses should be detectable in the intermediate IgG. Contaminated batches should be discarded.

The BWP recommended that a positive opinion may be issued on quality grounds, provided that the quality assurance and routine control measures as indicated above are implemented.

3. Toxicopharmacological aspects

Pharmacodynamics

The desired pharmacodynamic action of LeukoScan is the in vivo radiolabelling of blood granulocytes, granulocytes migrating to the site of infection and granulocytes at the site of infection.

The functional integrity of the antibody was demonstrated by its specificity for CEA. Certain antibodies against CEA that cross react with granulocytes have been proven to be specific for imaging of granulocytes in bone marrow and in sites of infection and inflammation. The molecule present on granulocytes that is reactive with Sulesomab has been shown to be NCA-90, in studies using HeLa cells transfected with cDNA genes that are known to be specific for CEA, NCA-90 and NCA-95. The company provided sufficient information on the NCA90 epitope and its relationship to CEA as well as information on the development of the product with particular reference to the specificity and CD classification of the molecular targets.

Specificity of LeukoScan is demonstrated by in vitro studies examining its relative binding capacity to granulocytes, monocytes and lymphocytes. Cross-reactivity with various human tissues was evaluated by immunostaining of cryostat frozen sections and fixed/paraplast-II embedded sections using indirect immunofluorescent staining with FITC-anti-murine Ig and assaying by fluorescent microscopy.

Human tissue staining studies performed with LeukoScan [^{99m}Tc] demonstrated staining of tissue granulocytes, blood granulocytes and the apical membranes of epithelial cells in the colon, rectum and

small intestine. Other organs known to be targeted by the agent are the kidneys and also the spleen and the bone marrow, which are both known to be rich in granulocytes. In vitro, a positive binding to lymphocytes up to 2-6% has been shown. The effect on lymphocyte function has not been determined.

It has also been shown that binding of LeukoScan to activated granulocytes is increased over binding of the agent to non-activated blood granulocytes, which would favour its reaction with granulocytes that are migrating to, or have accrued at sites of infection. In vitro assays have shown that it does not inhibit the function of granulocytes.

The possibility of LeukoScan causing anti-mouse antibodies exists, but probably not in high proportion on first exposure. Any pre-existing anti-mouse antibodies would be expected to react to some extent with LeukoScan.

Pharmacodynamic studies with LeukoScan have not been performed in animals because NCA-90 is not present on the granulocytes of animals, with the possible exception of the higher primates.

It is argued that drug interactions are unlikely to occur as the Fab' fragment is identical with the Fab domain of human IgG in blood which is present at much higher concentrations.

Pharmacokinetics

Two studies, one in mice and the other in hamsters, performed to study the biotransformation of ^{99m}Tc -LeukoScan in these species.

Size-exclusion HPLC analysis in sera of mice and hamsters showed that almost all the radioactivity was associated with Fab' and F(ab')_2 . The relative increase in ^{99m}Tc - F(ab')_2 is consistent with the different pharmacokinetic properties of Fab' and F(ab')_2 . F(ab')_2 has a significantly longer blood half-life because it is not filtered by the kidneys whereas Fab' is rapidly excreted by the kidneys.

After 1 hour of injection with ^{99m}Tc -LeukoScan the ^{99m}Tc products in the urine of hamsters was similar to mice, although in hamsters, considerably more radioactivity was associated with intact Fab' even at 6 hours post infusion. Biotransformation studies have also shown that ^{99m}Tc LeukoScan is stable in blood, demonstrating that the assay of ^{99m}Tc is a reliable surrogate of the biodistribution of the fragment. However the animal model, which does not express the epitope, the antibody is binding, and furthermore, there is no evidence that these biotransformation data are comparable to the studies done in humans.

Pharmacokinetic studies after repeated administration have not been performed because of the lack of the target antigen (NCA-90), the rapid transformation of the agent by the kidney and the short half-life of the radioisotope.

Autoradiography studies aimed at investigating the distribution in normal and pregnant animals were not performed because of the lack of NCA-90 in animals. Therefore such studies would not be expected to identify any cell type or structure with which the agent would specifically react.

Toxicity

Single dose toxicity: No toxic effects were seen in mice or rats after the animals were injected with more than 50 times the human ^{99m}Tc -LeukoScan dose.

Repeated dose toxicity: ^{99m}Tc -LeukoScan was repeatedly injected intravenously into rats and rabbits following a schedule designed to induce maximum allergic reaction to the agent. No treatment-related abnormalities in a range of parameters including haematology, clinical chemistry and histology, or toxicities were detected after repeated injections with doses exceeding the human dose by fifty-fold.

Reproductive toxicity: Animal reproductive toxicity studies have not been conducted. The use of LeukoScan in pregnancy is addressed in the SPC.

Mutagenic potential: No tests have been performed. The chemical constituents of the Fab' are the same as the chemical constituents present in human IgG. The chemical reducing agent stannous chloride, and other pharmaceutical excipients are already approved in IV preparations used for radioisotope imaging. ^{99m}Tc is known to be widely accepted in clinical practice as an in vivo radioimaging agent. The radionuclide emits only pure gamma radiation of a relatively low energy. Mutagenicity tests were therefore considered not to be necessary.

Carcinogenic potential: No studies have been performed for similar reasons stated above.

Local tolerance: No evidence of toxicity at the local site of injection was observed in either the single dose or repeated dose toxicity studies, with the exception of redness at the site of injection in some animals in the repeated dose toxicity study.

Immunotoxicity: The low dose of Fab'-SH used clinically is essentially non immunogenic as it lacks the Fc domain of IgG. The use of the monovalent Fab'SH fragment also eliminates potential direct immunopharmacotoxic effects that could result from the use of the intact antibody, i.e. complement activation or antibody dependent cellular cytotoxicity, both of which are Fc-mediated

Ecotoxicology: No assessment of the potential for environmental effects has been provided.

During the centralised procedure questions regarding the preclinical characterisation were answered comprehensively so that no issues were left open.

4. Clinical aspects

The localisation of the site and extent of an infectious process is very important to the clinical management of infection foci affecting musculoskeletal system, and can significantly influence the treatment (e.g., drainage, therapy with antibiotics, surgery etc.).

The anatomically orientated imaging modalities (conventional radiography, x-ray CT-scan, magnetic resonance imaging (MRI), ultrasonography) are extremely useful for the detection of focal sites of inflammation in the brain, in the thorax or intraabdominally.

For evaluating patients with focal inflammation after surgery or involving the musculo-skeletal system, these morphologically orientated procedures are less effective and very difficult to interpret after bone trauma or surgery.

If there is no morphologic alteration (early in the beginning of an inflammatory process) these methods can be completely normal despite severe clinical symptoms (e.g., fever of unknown origin). Radionuclide techniques, such as ^{111}In -WBC or ^{67}Ga -citrate imaging have been applied with relatively good success for detection of early inflammation. However, a number of problems are not solved when using this method, e.g., the long time between injection and imaging (mostly more than 24 hours), the complicated biodistribution (especially for ^{67}Ga), and the very time-consuming and complicated procedures for isolating granulocytes from the patients' blood when using ^{111}In -oxine imaging.

The gold standard in nuclear medicine imaging of infectious diseases using radiolabelled autologous white blood cells requires time-consuming ex-vivo blood handling techniques, special training and presents potential risk of infecting nuclear medicine personnel or even by infecting the patient (which has occurred by confusing the blood samples) (Becker et al., 1994).

The in-vivo labelling of leukocytes using monoclonal antibodies avoids the complicated isolation of granulocytes by labelling the cells with a $^{99\text{m}}\text{Tc}$ labelled monoclonal antibody in-vivo. Areas of concern using murine monoclonal antibodies include the development of human anti-mouse antibodies (HAMA), and altered biodistribution after repeated injection which could alter imaging and interfere with in-vitro immunoassays. To avoid this problem, chimeric or humanised or human antibodies are developed, lower doses of antibodies are used, and antibody fragments instead of intact IgG can be applied.

This in-vivo labelling of human granulocytes is a new development, but has been routinely applied, especially for imaging the bone marrow, in ten thousands of patients. The main advantage is that nuclear medicine methods are not invasive and have no pharmacodynamic effects. When using $^{99\text{m}}\text{Tc}$ labelled antibody fragments, the images are of higher quality due to the better count rates obtained when using $^{99\text{m}}\text{Tc}$ instead of ^{111}In or ^{67}Ga . The use of $^{99\text{m}}\text{Tc}$ also enables the application of SPECT, which makes the localisation more precise. The risk of damaging the granulocytes by separation procedures can be avoided, and also the risks which are inherent when manipulating with the blood or the cells of the patient.

Pharmacodynamics

Dose determining studies (see Table 1).

Data was pooled from 53 patients enrolled in 3 studies (01, 02, and 03).

It was observed that there was satisfactory sensitivity and specificity at doses ranging from 0.1 mg to 1.0 mg. Thus the SPC recommendation of 0.25 mg is considered to be reasonable.

Study 12 is a single group open label design, which was designed to investigate in patients with known abscesses:

- i) granulocyte function
- ii) safety and efficacy of LeukoScan [^{99m}Tc].

As far as granulocyte function is concerned, where the measurements of chemiluminescence were made on whole blood, it is argued that macrophages could also contribute to these responses and therefore the term “phagocyte function” is considered to be preferable.

Pharmacokinetics

Studies 01, 02, and 03, show that the pharmacokinetics and clearance observed with an investigational batch are satisfactory. Dosimetry demonstrates that doses to organs are within acceptable levels for radiopharmaceuticals.

Study no. 15 was designed to evaluate bioequivalence, dosimetry and in vivo stability of the 2 formulations of LeukoScan; the investigational and commercial. Bioequivalence in terms of AUC as defined in the protocol could not be adequately demonstrated between the 2 formulations. However, the mean blood clearance curves show close similarity between the 2 formulations of LeukoScan. It is thought unlikely that the differences between the 2 formulations would be of any clinical significance as radioimmunodiagnostic agents are generally employed on a single occasion.

Summary of clinical studies:

Study no.	Phase & study aim	Centres	Study design	Dose of radio-label (MBq), antibody (mg)	Patient no.
01 02 03	Phase I/II: Dose tolerance, pharmacokinetics	5	Single group open label uncontrolled	185-925 MBq, 0.1-1.0 mg	53
15	Phase I/II: Bioequivalence of 2 formulations	1	2 way double blind cross-over	740-1110 MBq, 0.25 mg	13
12 ongoing	Phase I: Evaluation of granulocyte function	1	single group, open label	555-925 MBq, 0.25 mg	5
07	Phase III: Diagnosis of osteomyelitis in patients with dia-betic foot ulcers	20	single group blinded, primary-standard-controlled	555-925 MBq, 0.25 mg	102
08	Phase III: Diagnosis of long bone osteomyelitis	19	single group blinded, primary-standard-controlled	555-925 MBq, 0.25 mg	130

Efficacy

Two major phase III trials in osteomyelitis were submitted.

Study 07 evaluated LeukoScan in the detection of osteomyelitis in 102 patients with diabetic foot ulcers.

Study 08 evaluated LeukoScan in 130 patients with suspected long bone osteomyelitis.

Studies 07 and 08 have many similarities in design and clinically diabetic foot ulcer patients generally have osteomyelitis in long bones. Hence a combined analysis of the efficacy data was performed with a total number of 175 evaluable patients.

LeukoScan is shown to be more sensitive (93.3% versus 80.6%-p=NS), but less specific (51.6 % versus 72.9% - p = NS) in diagnosing osteomyelitis in diabetic foot ulcer patients than in patients with other forms of long bone osteomyelitis a equivalent diagnostic accuracy. The same trend is observed in WBC imaging.

The global analysis confirms the efficacy of LeukoScan in osteomyelitis of long bones and associated with diabetic foot ulceration.

Efficacy end points were defined in terms of:

1. Diagnostic utility; where the primary endpoint was to compare LeukoScan imaging with bone biopsy histopathology and microbial culture and the secondary endpoint was the comparison of LeukoScan to conventional leukocyte imaging i.e. LeukoScan and white blood cell (WBC) scans are read at the site by the nuclear physician. Sensitivity, specificity, accuracy, positive predictive value (PPV) and negative predictive value (NPV) were measured.
2. Clinical benefit; defined as one for whom both the LeukoScan diagnostic procedure was correct and a diagnostic imaging procedure, a clinical management action (or both) was changed as result of LeukoScan interpretation. The clinical implication of diagnosing osteomyelitis is that intensive antibiotic treatment and surgery may be required.

LeukoScan was found to be equivalent to WBC scanning, with statistically superior sensitivity (95% vs. 84%), but lower specificity (55.6% vs. 63.0%). LeukoScan has advantages over WBC scanning in terms of convenience and lacks the potential for inadvertent administration of the wrong blood sample. Hence the indication for LeukoScan is justified.

It should be noted that comparing LeukoScan versus bone-scan, the sensitivity of the antibody scan was higher than for the bone scan in osteomyelitis in patients with diabetic foot ulcer: this could be possibly due to the infection being strictly limited to the bone marrow and therefore not inducing any detectable alteration in the bone structure.

Safety

The safety profile of LeukoScan is regarded as acceptable in relation to reported adverse events, vital signs and the lack of generation of Human Anti Mouse Antibodies (HAMA).

The fall in leukocyte and neutrophil numbers that occurs 24 hours following injection of LeukoScan is considered to be as within the normal range. An approximately 10% fall in blood leukocyte and neutrophil numbers was noted 24 hours following injection of LeukoScan. The overall minimal variations in WBC count seen in some of the clinical trials were of no clinical relevance, were likely due to change or "tendency to the mean" and were seen in uninfected individuals.

The risk of immunotoxicity of LeukoScan has been proved to be minimal because the Fab' cannot activate complement or effect antibody-dependent cytotoxicity, Fab' is monovalent and therefore cross-linking of antigen on cell surface is not possible, and finally the low dose of Fab' minimises any pharmacological effect of the mAb on granulocyte function. In vitro assays of granulocyte activation/function predicted that any direct immunotoxicity was unlikely.

The company committed to complete the studies on the LeukoScan binding to the human blood white cells submitting additional information on the labelling of lymphocytes as well as on the functional consequences of this binding to the lymphocytes.

Clinical data showed also that induction of human anti-murine antibodies (HAMA) with LeukoScan will be a very rare event.

5. Conclusion

During the centralised procedure questions regarding the preclinical characterisation were answered comprehensively so that no issues were left open.

Clinical safety of the product: No adverse effects definitely related to the antibody injection have been reported in 350 patients studied. Like all diagnostic nuclear medicine procedures, immunoscintigraphy uses only very small amounts of radiolabelled substances (tracer technique) and therefore adverse reaction rates are very low. No clinically significant changes in blood chemistry or hematology parameters were recorded. The use of an antibody fragment also reduces possible immunological reactions following the antibody injection. The incidence of HAMA is extremely low: In 228 patients tested, no human anti-mouse antibody reactions (anti-idiotypic as well as anti-isotypic responses) were found (very low immunogenicity of the product). The benefit risk ratio, which has been calculated (3.1:1) also demonstrated that the product is safe.

Clinical efficacy of the product: Clinical trials have been performed in 285 patients. Two major indications i) detection of bone infection in patients with diabetic foot ulcers (n = 102 patients) and ii) detection of long bone osteomyelitis (n = 130 patients) were studied in single-group, non-randomised prospective design trials. Diagnostic efficacy could be proven in 178 patients, which had bone biopsies and / or microbiological culture after immunoscintigraphy. 158 patients had comparative studies with white blood cell scans labelled by the conventional method (double radionuclide studies). 175 patients were assessed for diagnostic utility and clinical benefit.

The data presentation was clear and the data convincingly demonstrated that the product is useful for the claimed indications, statistically significant superiority of the product has been shown in comparison to the conventional diagnostic techniques (ex-vivo white blood cell labelling) used up to now in the clinic (diagnostic accuracy of LeukoScan 76.5 % vs. 70.9 % for conventional WBC labelling).

Of special importance (and from a clinical standpoint most essential) was the detailed analysis of clinical benefit results for the product. It was clearly demonstrated that anti-granulocyte antibody imaging reduced significantly the number of patients requiring other diagnostic procedures (85.4 % reduction), reduced the number of patients requiring antibiotic treatment (56.8 % reduction), and increased the need for surgery (61.3 % increase). Overall, 72 % of the patients experienced additional benefit and in 43 % an improvement of clinical outcome attributable to immunoimaging was recorded.

On this favourable risk/benefit assessment, the CPMP in their meeting on 15-16 October 1996 unanimously agreed on a favourable opinion for granting a marketing authorisation to LeukoScan in the following indication:

“LeukoScan is indicated for diagnostic imaging for determining the location and extent of infection/inflammation in bone in patients with suspected osteomyelitis, including patients with diabetic foot ulcers.

When bone scans are positive and imaging with LeukoScan is negative, infection is unlikely.

When a bone scan is negative, imaging with LeukoScan may rarely show a positive response and this may indicate early osteomyelitis.”