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# Recommendations of the Informal Consultation on Issues for Clinical Product Development for Human African Trypanosomiasis

Geneva, Switzerland

9–10 September 2004

World Health Organization

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# 1 Introduction

Researchers involved in clinical trials for the evaluation of new treatment modalities for human African trypanosomiasis (HAT), also known as 'sleeping sickness', face a number of challenges that are rarely, if ever, encountered in this combination in other diseases. A large number of these challenges are related to the fact that both the disease and the populations it affects are 'neglected', and there is thus no background of generally accepted—and ubiquitously feasible—diagnostic and treatment standards that usually form the basis for the planning and conduct of clinical evaluation of new treatment modalities for a disease.

Over the past years, interest in the evaluation and development of new treatment modalities has increased and it is thus timely to try to establish a common approach that will facilitate collaboration in the evaluation of new treatment modalities and/or facilitate comparison of data obtained by different groups.

This Informal Consultation on Issues for Clinical Product Development for Human African Trypanosomiasis was convened:

- To review and discuss the available data (which had been assembled and distributed in a briefing document, presented in the Appendix of the present report) with the very different organizations interested in the development of new treatment and diagnostic modalities for HAT; and
- To develop a consensus framework to guide the planning, conduct and analysis of clinical trials in the future in a way that would promote the acquisition of data that can be readily compared and used in meta-analysis.

The recommendations of the Informal Consultation were driven by the need for evaluation of the efficacy of new treatment regimens, but are in some cases directly applicable, in other cases easily adaptable, to the evaluation of new diagnostics.

The recommendations focus on the acquisition of data from clinical trials, since data acquired according to common criteria are the prerequisite for any meaningful comparison between the outcomes of different clinical trials.

With the objective of direct comparability of published data on drug efficacy in mind, recommendations for analysis and reporting of the efficacy of the treatment regimens under evaluation were also agreed upon.

The Informal Consultation did not discuss approaches to harmonization of the evaluation of the safety of new treatment regimens for HAT.

# 2 Identification of patients for clinical trials

## 2.1 Diagnosis

Only patients who are trypanosome-positive should be included in clinical trials.

- For evaluation of a patient's eligibility for inclusion in a clinical trial, trypanosomes should be searched for in blood *and* lymph-node aspirate (when punctionable lymph nodes are present) *and* cerebrospinal fluid (CSF).
- The most sensitive parasitological tests possible should be used as soon as possible after sample collection to retain maximum sensitivity. Repeated examinations (if possible, over several days) increase the probability of detecting trypanosomes.

## 2.2 Staging

To reduce the likelihood that the interpretation of the results of the clinical trial is confounded by factors other than those under investigation, the Informal Consultation recommended the use of the following criteria for staging and inclusion in clinical trials:

- Staging should be based on parasitological criteria and on the white blood cell (WBC) count in the CSF. The amount of protein in the CSF should not be taken into account for disease staging as this parameter is not specific for HAT and normal values vary with age.
- Patients eligible for enrolment in trials evaluating the treatment of first-stage (early or haemolymphatic) disease should have:
    - No trypanosomes in the CSF *and* a WBC count of ≤ 5 cells/µl CSF.
- Patients eligible for enrolment in trials evaluating treatment of second-stage (late or meningoencephalitic) disease should have:
    - WBC count > 20 cells/µl CSF (with or without trypanosomes in the CSF).
- Patients with the following characteristics should not be included in clinical trials aiming to recruit patients in the first or second stage of disease:
    - Patients with ≤ 5 WBC/µl CSF and trypanosomes in the CSF;
    - Patients with 6–20 WBC/µl CSF with or without CSF trypanosomes.

Some of these patients can be cured with first-stage drugs, while others need treatment with second-stage drugs. Patients with these characteristics thus have a response to a specific drug under evaluation that does not allow conclusions to be reached as to the efficacy of the drug against first-stage or second-stage HAT. Consequently, the inclusion of such patients in a clinical trial of drugs to treat either first- or second-stage HAT would compromise the assessment of the efficacy of the drug in its intended patient population.

Table 1 summarizes the consensus reached by the Informal Consultation regarding the inclusion of patients in clinical trials:

Table 1
**Diagnosis and staging-based criteria for inclusion of patients with HAT in clinical trials**

| Targeted patient population | Criteria |
|---|---|
| First stage | Trypanosome-positive blood and/or lymph<br>Trypanosome-negative CSF<br>≤ 5 WBC/µl CSF[a] |
| Second stage | Trypanosome-positive blood and/or lymph<br>Trypanosome negative or positive CSF<br>> 20 WBC/µl CSF[a] |

CSF: cerebrospinal fluid; HAT: human African trypanosomiasis; WBC: white blood cells

[a] When the CSF contains > 20 red blood cells/µl, the patient should be excluded from the trial unless a non-haemorrhagic CSF sample is obtained later.

## 2.3 Methodological considerations

### *2.3.1 Diagnosis of patients to be included in clinical trials*

- In order to provide complete parasitological baseline characteristics for the patients enrolled in a clinical trial, blood *and* lymph (when punctionable lymph nodes are present) *and* CSF should be examined for parasites at least once, even if the presence of trypanosomes has already been demonstrated in another body fluid.
- The most sensitive parasitological test possible should be used, i.e. mini-anion exchange centrifugation technique (mAECT) and/or capillary tube centrifugation (CTC) technique for blood examination, and modified single centrifugation or double centrifugation for CSF. Enlarged lymph nodes should be punctured for direct examination of lymph-node aspirate.
- Experience in the field has shown that individuals may be incorrectly categorized as parasitological positives. Confirmation of the presence of trypanosomes in each body fluid by a second staff member is recommended as mandatory for clinical trials to reduce the risk of including false parasitological positives in the trial and to obtain accurate data on baseline characteristics. Appropriate training of all personnel involved in a trial is essential.

### *2.3.2 Examination of CSF for staging*

- Lumbar puncture should be performed using sharp disposable needles.
- A volume of 5 ml of CSF should be collected. It is important that the first drops be discarded to avoid contamination of the CSF with red blood cells.
- Examination of the CSF for both trypanosomes and WBCs should be initiated not less than 5 minutes after collection and be completed within 30 minutes of CSF sampling, as CSF trypanosomes may die (and can thus no longer be detected) and CSF WBCs become deformed or disappear quite rapidly after collection of CSF.

- WBC counts:
    - — Before the WBC count, the CSF should be gently mixed to obtain a homogeneous suspension of cells. Türck solution should not be used for three reasons: (1) as Türck solution lyses red blood cells, it is difficult to assess whether a sample is haemorrhagic; (2) Türck solution may lyse trypanosomes that were present in the CSF; (3) normal CSF cell counts are already close to the detection limit for counting chambers and adding Türck solution increases the volume/dilutes the sample and lowers the accuracy of the cell count.
    - — When the CSF contains > 20 red blood cells/µl, the patient should be excluded from the trial unless a non-haemorrhagic CSF sample is obtained later.
    - — All types of WBCs should be included in the WBC count, not only lymphocytes. Use of standardized, single-use counting chambers is advised in order to avoid variations in the volume of CSF, which can occur with incorrectly mounted classical counting chambers. The counting chamber should never be filled straight from the lumbar puncture needle while the CSF is being collected, to avoid incorrect filling of the chamber.
    - — When the number of WBCs in a single CSF aliquot is < 30 per µl, the cell count should be performed on a second aliquot and the average of the two cell counts should be used for staging.
- The modified single centrifugation or the double centrifugation technique should be used to detect trypanosomes in the CSF. The former technique is preferable because of its rapidity, simplicity and lower workload. It has been reported that no difference in sensitivity has yet been demonstrated between the two methods (Miézan et al., 1994a; Miézan et al., 2000).

### *2.3.3 Evaluation of patients at end-of-treatment and at post-treatment follow-up*

- Within 1–2 days of the end of treatment (at the latest within 14 days of the end of treatment), an 'end-of-treatment (EoT)[1] evaluation'—parasitological examinations on blood and lymph (when punctionable lymph nodes are present)—should be performed. CSF examination at that time is indicated only for trials in which second-stage patients are enrolled and to determine the appropriate rescue treatment for first-stage patients with parasites in the blood and/or lymph.
- In trials in which first-stage patients are enrolled as well as in trials enrolling second-stage patients, parasitological examinations on blood and lymph (when punctionable lymph nodes are present) *and* CSF examination should be performed at each follow-up after the EoT evaluation.

[1] EoT evaluation, protocol-planned timing within 1–2 days, and at the latest within 14 days after the end of treatment.

- The full spectrum of parasitological tests, including the most sensitive tests available (CTC and/or mAECT) for blood, double or improved single centrifugation for CSF, should be used to increase the sensitivity of detection of non-responders and relapsing patients (Miézan et al., 1994a).Provisions for confirmation of trypanosome presence by a second staff member should be included in the protocol to reduce the risk of false positives.
- Given the relatively low prevalence of infection in most areas in which the disease is endemic, the probability that patients diagnosed as relapses are actually re-infections is likely to be small. Until validated field-usable methods allow an unambiguous distinction to be made between re-infection and relapse, no distinction will be made and all patients that are trypanosome-positive during post-treatment follow-up will be classified as relapses.

## 2.4 Collaboration between clinical trial teams and national control programmes for identification of patients for clinical trials

Because of the relatively low prevalence of HAT in most areas in which it is endemic, large numbers of people need to be screened (see Appendix, briefing document, section 3.2) to enrol the required number of patients.

Effective detection of patients for clinical trials is thus best carried out via close collaboration between the clinical-trial team and the national control programme and/or the nongovernmental organization conducting HAT control in the area where the trial is taking place. Such collaboration requires considerable flexibility from the collaborating groups, e.g. mobile screening teams may need to change their originally scheduled screening tours and target villages as close as possible to the clinical study centre to facilitate follow-up after treatment. On the other hand, the clinical trial team needs to be sensitive to the mobile team's capacity and experience.

During discussions by the clinical-trial team and the mobile-screening team regarding the criteria for referral or transport of patients to the clinical trial centre for further evaluation, factors such as the local prevalence of disease need to be taken into account. To what extent patients screened by the local mobile team are further evaluated by that team or in the clinical trial centre will depend on the capacity of the mobile team as well as on the capacity of the clinical trial centre. The clinical trial should not have a negative impact on the normal functioning of the centre and care for other patients. Whenever possible, a special clinical-trial team should assist the local mobile team.

The card agglutination test for trypanosomiasis (CATT) on whole blood is nowadays applied by all control programmes for screening of the population in areas in which *Trypanosoma brucei gambiense* is endemic. In order not to overburden the screening teams, only those individuals who have a positive CATT result at a serum dilution of 1:4 or higher should undergo parasitological examinations. The CATT is not currently used for screening in areas in which *T. b. rhodesiense* is endemic.

These requirements need to be taken into account during preparation and budgeting of the clinical trial.

# 3 Evaluation of new surrogate markers

There is an urgent need for further validation of existing surrogate markers and for the identification of new markers that could allow the sensitivity of diagnosis to be increased, the evaluation of the treatment response to be improved and/or the post-treatment follow-up period to be reduced.

Consequently, surrogate markers (e.g. IgM titres, HAT-specific antibody titres) should be evaluated whenever possible in the context of clinical studies designed for other purposes, e.g. clinical trials of new treatment modalities.

The timing of the test-of-cure visit (ToC)[2] and the definitions of categories for disease stage and response, as proposed in the present document (see sections 4 and 5), should be reviewed and refined when appropriate, as validated surrogate markers become available.

The requirement for lumbar puncture for the reliable diagnosis of second-stage disease and, in particular, the requirement for repeated lumbar punctures for the timely diagnosis of relapse, is a major problem for patient care, disease control and clinical trials. Surrogate markers that have the potential to allow staging and detection of relapse without lumbar puncture, whether for use in disease control or 'only' for clinical trial use, should be given priority for evaluation and validation.

# 4 Time-point for final assessment of efficacy (ToC visit)

In HAT control programmes, the final assessment of the efficacy of treatment for HAT is currently scheduled at 24 months after treatment; this is based on the recommendations of the 1986 meeting of the WHO Expert Committee on Epidemiology and Control of African Trypanosomiasis regarding the minimum follow-up period required before treatment can be considered successful. The data on which this recommendation was based are not provided in the WHO Expert Committee report (WHO Expert Committee on Epidemiology and Control of African Trypanosomiasis, 1986).

Requirements for the assessment of efficacy in clinical trials are different from those for efficacy assessment in HAT control programmes. Prior experience has shown that quantification of the efficacy of a treatment modality 24 months after treatment is not as robust as would be desirable, since at that time data on patients' status are missing for between 20% and 80% of treated patients. Therefore, the Informal Consultation discussed whether another time-point after treatment would provide a more reliable assessment of efficacy. Data relevant for this discussion were assembled by the preparatory committee who prepared the briefing document:

- Data on time of relapse after treatment (see Table A1.13);
- Data on patient follow-up rates at different times after treatment (see Table A1.14).

---

[2] Test-of-cure evaluation (final efficacy assessment), protocol-planned timing 18 months after treatment

On the basis of a discussion of the proportion of treated patients diagnosed as relapsed at different time-points after treatment, the associated follow-up rates and the reliability of the data, the following conclusions were reached:

- The categorized data assembled in the briefing document suggest that at least 70% of relapses known to take place between 24 and 36 months after treatment have already occurred within 18 months after treatment, and that between at least 40% and 100% of relapses have already occurred within 12 months after treatment.
- The variability in the percentage of patients diagnosed as relapsed at certain time-points after treatment is likely to be related to a number of factors including, but not limited to:
    - The number of follow-up investigations per patient and the percentage of patients who have follow-up examinations at different time-points after treatment;
    - The times after treatment at which the follow-up examinations took place, i.e. post-treatment examinations at a sufficient number of time-points after treatment to allow detection of relapse at a time close to when relapse first becomes detectable;
    - Biological factors (e.g. differences in inclusion criteria, disease status at baseline);
    - Technical aspects of follow-up (methods employed, experience of investigators and technicians);
    - The criteria for relapse used by different control programmes and in different studies;
    - Differences in drugs and drug regimens used.
- To test this hypothesis and potentially arrive at a more accurate estimate of time of relapse after treatment, an analysis of the raw data from studies/treatment programmes with acceptable and comparable quality of patient follow-up and data collection needs to be conducted, with stratification for factors that may influence time of relapse and time at which relapse was diagnosed. Organizations that have recently performed clinical trials (e.g. Médecins Sans Frontières, Malteser and the Swiss Tropical Institute) will provide raw data to be analysed. An informal working group will be formed to further discuss requirements for the data analysis.
- Post-treatment follow-up data are available for a much higher percentage of patients at 18 months than at 24 months, even in studies with active follow-up (see Table A1.14). The vast majority of patients who relapse within 24 months of treatment have already relapsed within 18 months (see Table A1.13). Therefore, efficacy assessment based on 18 months post-treatment follow-up data would provide a good estimate of the efficacy of the drug under investigation, in particular in comparative studies. Consequently, 18 months can at this stage be recommended as the time for the final efficacy assessment (test-of-cure visit) in clinical studies.

- The percentage of patients with follow-up data is substantially higher at 12 months after treatment than at 18 months after treatment (see Table A1.14). Furthermore, the available data suggest that, in most case series or trials, the majority of relapses occurred within 12 months after treatment (see Table A1.13). Consequently, for a final efficacy assessment, post-treatment data acquired at 12 months may provide an equally good estimate of the efficacy of the drug under investigation as the data acquired at 18 months. The data in the Appendix, Tables A1.13 and A1.14 were obtained by different organizations using different protocols for data acquisition and analysis. The participants in the Informal Consultation felt that this limited the conclusions that could be drawn from these data. It was recommended that the raw data available from the different organizations be analysed and subsequently reviewed to determine whether they supported a recommendation for a final efficacy assessment based on follow-up data acquired at 12 months.
- To ensure the timely diagnosis of relapse, patients should have follow-up evaluations at least at the end of treatment (including CSF examination for second-stage patients), and 3 or 6, 12 and 18 months after treatment. The choice of 3 or 6 months follow-up will depend on prior knowledge concerning the efficacy of the drug or treatment regimen under evaluation. To ensure data comparability between trials, clinical studies requiring assessments at other time-points should add these time-points, rather than replace the recommended evaluations at 3 or 6, 12 and 18 months after treatment.
- To increase the number of patients with follow-up data acquired 18 months after treatment, it is recommended that the study protocol contains plans to continue efforts for several months after the 18 months post-treatment follow-up time-point has passed, to localize and evaluate patients who did not present for the 18 months assessment and were not previously diagnosed as relapsed.

# 5 Patient evaluation and criteria for assessment of efficacy

The limitations of the current methods for diagnosis and staging of HAT, in particular the low sensitivity with which trypanosomes are detected in blood, lymph and CSF, significantly affect patient follow-up, the decision on when to initiate rescue treatment and thus the assessment of the efficacy of treatment modalities. The reluctance or refusal of patients to come back for follow-up visits and undergo repeated lumbar punctures further aggravates these difficulties, since no data at all or no CSF data (presence or absence of trypanosomes, WBC count, or other CSF surrogate markers) may be available for the assessment of the patients' state of health.

These factors have certainly contributed to the use of different criteria to determine relapse by different investigators (see Appendix, briefing document, section 2.2) and to the introduction of a category of 'suspected relapse' and a category of 'patients requiring close follow-up' in many trials for patients with clinical signs and/or CSF WBC counts suggesting a relapse but without parasitological confirmation.

The Informal Consultation discussed experience in the field, including experience with the reluctance of patients to undergo repeated lumbar punctures, the requirement for criteria suitable to achieve the right balance between treatment for relapse of a deadly disease as soon as necessary and not exposing patients to toxic treatments unnecessarily, as well as the requirement for a scientifically sound assessment of the efficacy of a treatment modality, and came to the following conclusions:

1 For clinical trials, an elaborate classification system is required for patients during follow-up.

2 There are few hard data that could serve as the basis for objective, data-based criteria for the determination of response status in the absence of parasitological evidence. In the absence of such data, a complete set of objective criteria that cover the whole range of patient characteristics cannot be provided. Consequently, the criteria in the following tables refer to the investigator's judgement for any decisions for which objective criteria are not available.[3]

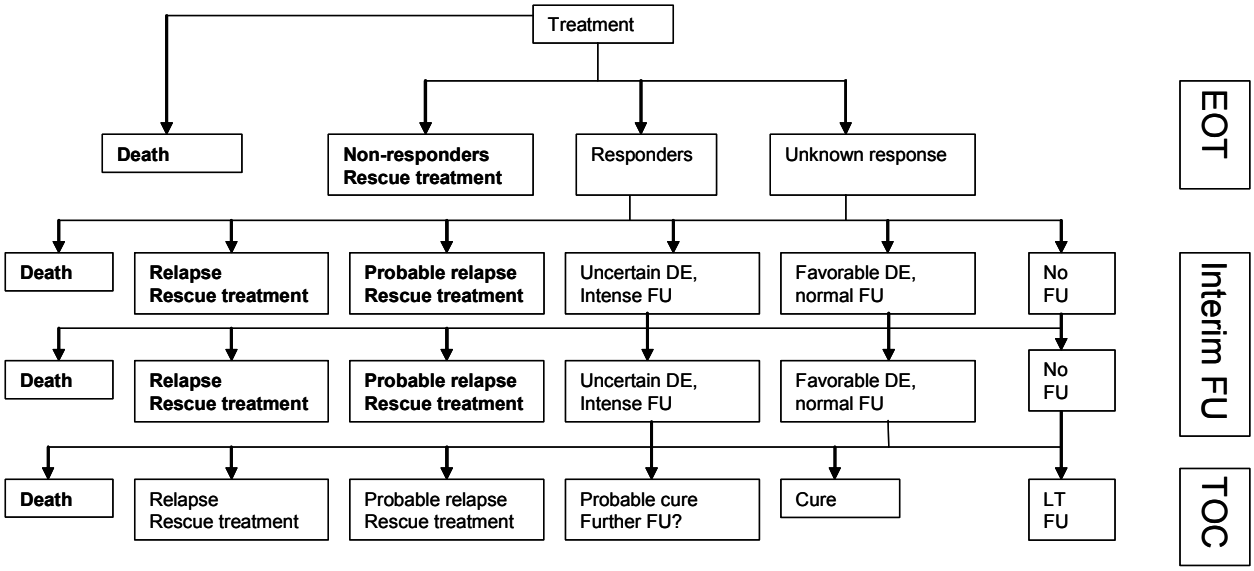
3 The lack of data on which to base objective criteria for the categorization of response status is one example reflecting the need for all institutions conducting clinical trials or treatment programmes to assemble comprehensive databases on the clinical and laboratory characteristics of all patients at each follow-up. Eventually these can be pooled and analysed for data on which recommendations for objective criteria can be based.

4 Data to date suggest that an increase in the CSF IgM titre precedes the increase in CSF WBC counts and occurs before trypanosomes can be detected in the CSF. Consequently, an increase in CSF IgM titre may have prognostic value for relapse (Lejon et al., 2002). Since CSF IgM titre has not yet been validated as a surrogate marker/predictor of relapse, it should not be used to make treatment decisions. However, the data available do support the use of changes in CSF IgM titre for the identification of patients who should be monitored closely by the standard methods for detection of relapse (see patients with 'uncertain evolution' of disease).

Figure 1 provides an overview of the recommended classification of patients at different times after treatment: end of treatment (EoT), during interim treatment follow-up (interim follow-up) and at the test-of-cure (ToC) at the last follow-up visit. The criteria for each classification are provided in sections 5.1 and 5.2 for patients with first-stage and second-stage HAT, respectively.

[3] The judgment of the investigator, the criterion recommended here, could be replaced in a specific protocol by criteria agreed upon between sponsor(s) and investigator(s).

Figure 1
**Terminology for assessment of the patient after treatment**




| | |
|---|---|
| **Bold type** | End-point reached before ToC visit |
| **DE** | Disease evolution |
| **EoT** | End-of-treatment evaluation within 1–2 days, latest 14 days, after the end of treatment |
| **FU** | Follow-up |
| **Interim FU** | Interim follow-up: at 3 or 6 (depending on prior knowledge about the efficacy of the drug or treatment regimen under evaluation) and 12 months after treatment |
| **LP** | Lumbar puncture |
| **LT FU** | Lost to follow-up |
| **ToC** | Test-of-cure evaluation (final efficacy assessment) 18 months after treatment |

## 5.1 Patients with first-stage HAT

### *5.1.1 Evaluation of first-stage patients at the end of treatment*

Within 1–2 days and at the latest within 14 days after the end of treatment, the protocol should plan for patients to be assessed for the presence of trypanosomes in the blood and lymph, when lymph-node aspirate examination is feasible. CSF examination is not regarded as necessary, except for non-responders who need lumbar puncture to determine the stage of the disease and thus appropriate rescue treatment.

Depending on survival of the patient during treatment and outcome of the EoT evaluation, as applicable, patients will be classified as summarized in Table 2.

Table 2
**Criteria for classification of first-stage patients at the EoT evaluation**

| Category | Patient characteristics/Criteria | Action |
|---|---|---|
| Death | See section 5.1.4 for more details | |
| Non-responders | Patients with evidence of trypanosomes in blood and/or lymph | They will receive rescue treatment as indicated by the stage of the disease determined at the EoT evaluation |
| Responders | Patients with no evidence of trypanosomes in blood and/or lymph | They will be scheduled for the next protocol planned follow-up visit |
| Unknown response | Patients for whom no EoT evaluation data are available | All attempts should be made to evaluate these patients as soon as possible |

EoT: end-of-treatment

### *5.1.2 Evaluation of first-stage patients during interim follow-up*

To ensure timely diagnosis of relapse, interim follow-up evaluations, including lumbar puncture, should be performed 3 or 6 months (depending on prior knowledge concerning the efficacy of the drug or treatment regimen under evaluation) and 12 months after treatment. Additional follow-up visits should be scheduled if indicated by the protocol or clinically (e.g. when the patient is classified as having 'uncertain evolution' of the disease).

Depending on the survival of the patient during follow-up, availability of follow-up evaluation data and outcome of the evaluations, it is recommended that patients be classified as shown in Table 3.

### *5.1.3 Test-of-cure evaluation for first-stage patients*

At the test-of-cure visit 18 months after treatment, parasitological examination of the blood and lymph (when feasible) and CSF examination should be performed and patients classified as shown in Table 4.

Table 3

**Criteria for classification of first-stage patients (with positive or unknown response at EoT evaluation) during interim follow-up visits**

| Category | Patient characteristics/Criteria | Action |
|---|---|---|
| Death | See section 5.1.4 for more details | |
| Relapse | Patients in whom trypanosomes have been detected in any body fluid | Rescue treatment as per study protocol |
| Probable relapse | Patients without parasitological evidence of relapse in any body fluid AND<br>with > 20 WBC/µl CSF AND<br>whose CSF is not haemorrhagic and WBC count is unlikely to be due to a disease other than HAT<br>Patients without parasitological evidence of relapse in blood and lymph<br>who refuse lumbar puncture OR whose CSF sample is haemorrhagic without trypanosomes AND<br>who in the opinion of the investigator require immediate rescue treatment based on a marked deterioration of their clinical condition unlikely to be due to another disease than HAT | Rescue treatment as per study protocol.<br>Note: all possible efforts should be undertaken to convince a patient suspected of relapse to undergo lumbar puncture before classification as 'probable relapse' and thus the decision to administer rescue treatment |
| Uncertain evolution | Patients without parasitological evidence of relapse in any body fluid AND<br>with 6–20 WBC/µl CSF in a non-haemorrhagic sample<br>Patients without parasitological evidence of relapse in blood and lymph<br>who refuse lumbar puncture OR whose CSF sample is haemorrhagic without trypanosomes AND<br>who do not present with a marked clinical deterioration compared to the previous evaluation AND<br>who in the opinion of the investigator should have an additional follow-up investigation in 1 or 3 months<br>In studies that determine the CSF IgM titer:<br>Patients without parasitological evidence of relapse in blood and lymph AND<br>with < 20 WBC/µl CSF AND<br>with an at least fourfold increase in the LATEX/IgM CSF titer compared with the last evaluation (only valid for CSF that is not haemorrhagic) | Additional follow-up after 1–3 months with clinical evaluation and evaluation of blood, lymph and CSF |
| Favourable evolution | Only patients who undergo lumbar puncture and whose CSF sample is not haemorrhagic can be classified as 'favourable evolution' if they are:<br>Patients with ≤ 5 WBC/µl CSF and no parasitological evidence of relapse | These patients will be scheduled for the next protocol planned follow-up visit |

CSF: cerebrospinal fluid; EoT: end-of-treatment; HAT: human African trypanosomiasis; Ig: immunoglobulin; WBC: white blood cell

Table 4

**Criteria for classification of first-stage patients (with positive or unknown response at EoT and not diagnosed as relapsed or probably relapsed at an interim follow-up[a] assessment) at 18 months ToC visit**

| Category | Patient characteristics/Criteria | Action |
|---|---|---|
| Death | See section 5.1.4 for more details | |
| Relapse | Patients in whom trypanosomes have been detected in any body fluid. | Rescue treatment as per study protocol. |
| Probable relapse | Patients without parasitological evidence of relapse AND<br>with > 20 WBC/µl CSF AND<br>whose CSF is not haemorrhagic and WBC count is unlikely to be due to a disease other than HAT.<br>Patients without parasitological evidence of relapse in blood and lymph<br>who refuse lumbar puncture OR whose CSF is haemorrhagic without trypanosomes AND<br>who in the opinion of the investigator require rescue treatment, because they present a marked deterioration of their clinical condition that is unlikely to be due to another disease than HAT | Rescue treatment as per study protocol<br>Note: all possible efforts should be undertaken to convince a patient suspected of relapse to undergo lumbar puncture prior to classification as 'probable relapse' and thus the decision to administer rescue treatment |
| Probable cure | Patients without parasitological evidence of relapse AND<br>with 6–20 WBC/µl CSF in a non-haemorrhagic sample<br>Patients without parasitological evidence of relapse in blood and lymph AND<br>who refuse lumbar puncture OR whose CSF is haemorrhagic AND<br>who in the opinion of the investigator do not require rescue treatment because their clinical condition is satisfactory or their symptoms are attributed to a disease other than HAT | Further follow-up at 24 months is not required for clinical trial data purposes<br>Depending on the prior follow-up history of the patient, the investigator may decide to follow the patient further at the clinical trial centre or refer him to the nearest national control programme facility for routine follow-up 24 months after treatment |
| Cure | Only patients who undergo lumbar puncture and whose CSF sample is not haemorrhagic can be classified as cured if they are:<br>Patients with ≤ 5 WBC/µl CSF and no parasitological evidence of relapse. | |

CSF: cerebrospinal fluid; EoT: end-of-treatment; HAT: human African trypanosomiasis; ToC: test-of-cure; WBC: white blood cell

[a]Interim follow-up examinations for efficacy and safety are conducted between the EoT evaluation and the ToC evaluation, protocol planned timing at 3 or 6 (depending on prior knowledge about the efficacy of the drug) and at 12 months after treatment

### *5.1.4 Deaths*

Patients who died during treatment or during follow-up will be categorized based on the likely or definite cause of death:

- Human African trypanosomiasis;
- Adverse events regarded by the investigator as possibly, probably or definitely related to treatment for HAT;
- Causes unrelated to HAT or treatment for HAT;
- Unknown causes.

Before classification of a patient as having died due to unknown causes, attempts should be made to determine the cause of death. In the case of death during follow-up, interviews of family members and neighbours or local health-care workers (sometimes referred to as 'oral autopsy') may provide the likely cause of death. 'Suggestive questioning' should be avoided. Standardization of techniques for oral autopsy between clinical trials should be discussed.

## 5.2 Patients with second-stage HAT

### *5.2.1 Evaluation of second-stage patients at EoT*

Within 1–2 days and at the latest within 14 days after the EoT, the protocol should plan for the patients to be assessed for the presence of trypanosomes in the blood, lymph (when feasible) and CSF (EoT evaluation). Because CSF WBC counts may not have normalized at that time, they should not be taken into account for classification as responder or non-responder (Dumas & Girard, 1978). Depending on survival of the patient during treatment and outcome of the EoT evaluation, as applicable, patients will be classified as shown in Table 5.

Table 5
**Criteria for classification of second-stage patients at the EoT evaluation**

| Category | Patient characteristics/Criteria | Action |
|---|---|---|
| Death | See section 5.1.4 for more details | |
| Non-responders | Patients with evidence of trypanosomes | They will receive rescue treatment as per study protocol |
| Responders | Patients with no evidence of trypanosomes | They will be scheduled for the next follow-up visit |
| Unknown response | Patients for whom no data on EoT evaluation are available | All attempts should be made to evaluate these patients as soon as possible |

EoT: end-of-treatment

### *5.2.2 Evaluation of second-stage patients during interim follow-up*

To ensure timely diagnosis of relapse, interim follow-up evaluations, including lumbar puncture, should be performed at least 3 or 6 months (depending on prior knowledge concerning the efficacy of the drug or treatment regime under evaluation) and 12 months after treatment. Additional follow-up visits should be scheduled if indicated by the protocol or clinically (e.g. when the patient is classified as having 'uncertain evolution' of the disease).

Depending on the survival of the patient during follow-up, availability of follow-up data and outcome of the evaluations, patients will be classified as shown in Table 6.

These recommendations, even more than those for first-stage patients, leave the establishing of criteria for diagnosing patients with 'probable relapse' (resulting in rescue treatment) or 'uncertain evolution' of the disease (resulting in close follow-up) to those responsible for the study protocol, i.e. dependent on sponsor and investigator agreement (see section 5).

The rationale for these recommendations is that there are currently not sufficient data on the relationship between WBC counts (or other surrogate markers of infection) and parasitological and clinical evidence of cure and relapse. Several studies have been recently completed or are about to be completed that have collected relevant data. Once an analysis of these data has been completed, the data and data analysis will be reviewed by experts to see whether they provide a sufficient basis for evidence-based criteria for patient classification during follow-up.

### *5.2.3 ToC evaluation of second-stage patients*

At the ToC visit 18 months after treatment, parasitological examination of the blood, lymph (when feasible) and CSF should be performed and patients classified as shown in Table 7.

Table 6

**Criteria for classification of second-stage patients (with positive or unknown response to treatment at EoT) during interim follow-up[a] visits**

| Category | Patient characteristics/Criteria | Action |
|---|---|---|
| Death | See section 5.1.4 for more details. | |
| Relapse | Patients in whom trypanosomes have been detected in any body fluid at any follow-up visit. | Rescue treatment as per study protocol |
| Probable relapse | Patients without parasitological evidence of relapse in any body fluid AND<br>who, in the opinion of the investigator, require rescue treatment, because they present a marked deterioration of their clinical condition unlikely to be due to another disease than HAT and/or they have CSF WBC counts suggestive of relapse in the investigator's assessment and/or their CSF was haemorrhagic. | Rescue treatment as per study protocol<br>Note: all possible efforts should be undertaken to convince a patient suspected of relapse to undergo lumbar puncture prior to characterization as 'probably relapsed' and thus the decision to administer rescue treatment |
| Uncertain evolution | Patients without parasitological evidence of relapse in any body fluid AND<br>who, in the opinion of the investigator, should have a follow-up investigation in 1–3 months because they present e.g.:<br>with a deterioration of their clinical condition that might or might not be due to HAT AND/OR<br>they have a rising CSF WBC count that might or might not be due to HAT, in the investigator's assessment | Additional follow-up after 1–3 months with evaluation of blood, lymph and CSF |
| Favourable evolution | Only patients who undergo lumbar puncture and whose CSF is not haemorrhagic can be classified as 'favourable evolution' IF they are<br>Patients without parasitological evidence of relapse AND<br>≤ 20 WBC/µl CSF OR<br>> 20 WBC/µl CSF AND decreased from previous values | These patients will be scheduled for the next follow-up visit as applicable within the regular 3 or 6, 12 and 18 months follow-up schedule in the protocol |

CSF: cerebrospinal fluid; EoT: end-of-treatment; HAT: human African trypanosomiasis; ToC: test-of-cure; WBC: white blood cell

[a] Interim follow-up examinations for efficacy and safety are conducted between the EoT evaluation and the ToC evaluation, protocol planned timing at 3 or 6 (depending on prior knowledge about the efficacy of the drug) and at 12 months after treatment

#### *5.2.4 Deaths*

Patients who died during treatment or during follow-up will be categorized on the basis of likely or definite cause of death, as described in section 5.1.4.

# 6 Quantification of efficacy

Ideally, all subjects within a clinical trial or treatment protocol/series should comply with all inclusion/exclusion criteria, be treated exactly as planned in the protocol and have a complete set of follow-up data. In practice, this is never the case, and thus the question arises as to which patients and which data to include in a particular analysis. To ensure an unbiased analysis of the data, the analyses to be conducted should be clearly specified before initiation of the study, i.e. in the study protocol.

The major sources of potential bias discussed during the meeting of the Informal Consultation included criteria for:

- Inclusion or exclusion of patients in particular analyses;
- How to deal with missing follow-up or final efficacy evaluation data;
- How to include those patients in the analysis of efficacy at a specific time-point during the study whose follow-up data had not been obtained within the time periods planned in the protocol.

In addition, the Informal Consultation discussed the type of efficacy analyses to be conducted and the definition of a common nomenclature.

Analyses and reporting of efficacy according to the nomenclature and criteria recommended below would facilitate the direct comparison of data reported for different clinical studies and treatment protocols/series.

The discussions and the resulting definitions and recommendations for data analysis were based on the guidelines for statistical analysis (http://www.ich.org/LOB/media/MEDIA485.pdf) issued by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH, www.ich.org).

## 6.1 Analysis populations

Definition of criteria for the inclusion/exclusion of patients for specific analyses, i.e. definition of ‘analysis sets’ or ‘analysis populations’, should be included in the protocol.

The nomenclature outlined in Table 8 is commonly used and was recommended by the Informal Consultation.

Table 7

**Criteria for classification of second-stage patients at the ToC visit at 18 months**

| Category | Patient characteristics/Criteria | Action |
|---|---|---|
| Death | See section 5.1.4 for more details | |
| Relapse | Patients in whom trypanosomes have been detected in any body fluid | Rescue treatment as per study protocol |
| Probable relapse | Patients without parasitological evidence of relapse in any body fluid AND<br>with > 20 WBC/µl CSF AND whose WBC count cannot be explained by a disease other than HAT<br>Patients without parasitological evidence of relapse in blood and lymph<br>who refuse lumbar puncture OR whose CSF is haemorrhagic without trypanosomes AND who in the opinion of the investigator require rescue treatment because a marked deterioration of their clinical condition unlikely to be due to another disease than HAT | Rescue treatment as per study protocol<br>Note: all possible efforts should be undertaken to convince a patient suspected of relapse to undergo lumbar puncture prior to classification as 'probable relapse' and thus the decision to administer rescue treatment |
| Probable cure | Patients without parasitological evidence of relapse in blood and lymph AND<br>who refuse lumbar puncture OR whose CSF sample is haemorrhagic without trypanosomes AND<br>whose clinical condition is satisfactory OR<br>whose clinical status is unlikely to be due to HAT | Further follow-up at 24 months is not required for clinical trial data purposes.<br>Depending on the prior follow-up history of the patient, the investigator may decide to follow the patient further at the clinical trial centre or refer him to the nearest national control programme facility for routine follow-up 24 months after treatment |
| Cure | Only patients who undergo lumbar puncture and whose CSF is not haemorrhagic can be classified as cured if they are:<br>Patients with ≤ 20 WBC/µl CSF and no parasitological evidence of relapse | |

CSF: cerebrospinal fluid; HAT: human African trypanosomiasis; ToC: test-of-cure; WBC: white blood cell

Criteria for defining analysis sets for efficacy populations discussed and recommended are provided in Table 9. Considering that in the first clinical trial of a new drug or new drug combination, the percentage of subjects who were parasite-free at the EoT may be the primary efficacy variable, a ‘modified full analysis set’ has been defined.

The efficacy parameters to be calculated for the analysis sets are described in Table 14.

Table 8
**Nomenclature for analysis sets**

| Analysis set | Comments |
|---|---|
| **Full analysis set, ‘intention-to-treat’ population** | The 'full analysis set' includes ideally all subjects randomized in a clinical trial (or treated within a treatment protocol/case series).<br>Exclusion of randomized subjects is acceptable only under circumstances where there is no danger of biasing the analysis outcome through this exclusion (e.g. the patient did not receive a single dose of treatment, or there are no follow-up data available that would allow the effect of the drug in that patient to be assessed).<br>The ‘intention-to-treat' analysis is regarded as the best way to assess the effect of a treatment policy, i.e. as an analysis that approximates the effect of treating according to a specific treatment policy in ' real life'. Consequently, for the 'full analysis set' or 'intent-to-treat' analysis, subjects are analysed as if they had received the treatment they were randomized to even if they received only a single dose of treatment, or actually received another treatment than the one to which they were randomized. |
| **Per-protocol set, ‘per-protocol’ population** | The 'per-protocol set' ideally includes subjects who have been enrolled and treated as planned in the protocol and for whom final efficacy data are available (i.e. subjects who reached a protocol defined end-point (e.g. discontinuation of treatment owing to treatment-related adverse events, death, relapse) before the end of treatment or before the end of the protocol-defined follow-up period (see boxes in bold type in Figure 1) and patients for whom efficacy data at the test-of-cure visit are available).<br>In some cases a 'per-protocol set' may be defined up-front, that also includes patients who have received only a pre-specified minimum amount of treatment (expected to have the minimum level of efficacy that the drug should have to perform its planned role in patient management/disease control) but not the full planned treatment, and/or patients who did not comply with specific requirements in the protocol (e.g. certain inclusion/exclusion criteria, concomitant treatment, concomitant diseases). This analysis set can be referred to as the 'modified’ per-protocol set to distinguish it from the 'ideal' per-protocol set.<br>The results of the 'per-protocol' analysis will allow conclusions as to the validity of the hypothesis underlying a clinical trial, i.e. that the drug under investigation, if administered as planned to a specific type of patient (defined via the inclusion and exclusion criteria in the protocol) will have a certain efficacy (with the level of efficacy expected (e.g. percentage of subjects cured, or superior to the comparator drug, or non-inferior to the comparator drug) specified in the protocol, usually in the sample size justification. |
| **Safety analysis set** | The 'safety analysis set' includes all subjects who received at least one dose of the drug.<br>Including all subjects who received at least one dose of the study drug allows the safety profile of the drug to be fully characterized among the patients being treated, including adverse events which result in treatment discontinuation after only one or few doses (e.g. allergies, other symptoms of intolerance). |

The defined minimum amount of treatment required for inclusion in the per-protocol and the modified-full-analysis population is drug-specific. For currently available drugs used as comparators, the minimum amount of treatment (to be specified in the protocol) is provided in Table 10.

Table 9
**Analysis sets for efficacy analysis[a]**

| Analysis set | Patients included |
|---|---|
| **Full analysis set (intention-to-treat population)** | All patients enrolled in the study<br>who received at least one dose of study medication<br>AND<br>who died during treatment or were non-responders OR for whom efficacy evaluation data at the test-of-cure visit or a protocol defined earlier time-point are available. |
| **Per-protocol set** | All patients enrolled in the study<br>in compliance with the inclusion/exclusion criteria<br>AND<br>who received at least one dose of study medication AND died during treatment or were non-responders OR for whom treatment was discontinued because of treatment-related adverse events<br>OR<br>who received the protocol-defined treatment AND<br>who reached one of the protocol-defined end-points (death, non-responder, relapse, probable relapse) before the ToC[b] or have a ToC visit assessment. |
| **Modified per-protocol set** | All patients enrolled in the study<br>with parasitologically confirmed infection<br>AND<br>who received at least one dose of study medication AND died during treatment or were non-responders OR were discontinued from treatment for treatment-related adverse events<br>OR<br>who received a defined minimum amount of treatment AND who reached one of the protocol defined end-points (death, non-responder, relapse, probable relapse) OR for whom efficacy evaluation data at the ToC visit or a protocol defined earlier time-point are available |
| **Safety-analysis set** | All patients enrolled in the study who received at least one dose of study drug |

ToC: test-of-cure

[a] For trials with other efficacy end-points (e.g. trials evaluating a new drug or new drug combination for the first time), these criteria need to be adapted accordingly.

[b] ToC evaluation (final efficacy assessment), protocol-planned timing 18 months after treatment

Table 10
**Minimum amount of treatment required for inclusion in the per-protocol analysis population**

| Drug | Standard treatment regimen | Minimum amount of treatment for per-protocol population |
|---|---|---|
| Melarsoprol for *T. b. gambiense* | 2.2 mg/kg per day IV x 10 days | 8 days of treatment without interruption or up to one treatment interruption for ≤ 2 days |
| Melarsoprol for *T. b. rhodesiense* | Different schedules | Decided by individual investigators until further field experience is available for evaluation |
| Eflornithine | 400 mg/kg per day IV x 14 days | 12 days of treatment without interruption or up to two treatment interruptions for up to two successive doses |
| Pentamidine | 4 mg/kg per day IM x 7 days | Up to one treatment interruption of ≤ 2 days |
| Suramin | Different schedules | Decided by individual investigators until further field experience is available for evaluation |

IM, intramuscular; IV, intravenous

## 6.2 Time windows for assigning patient follow-up data to times after treatment, for efficacy analysis

A protocol will usually plan for a specific action/evaluation to take place within a defined window around the 'ideal' time after treatment (for example the 3-month follow-up evaluation should be conducted at 3 months ± 1 week) (see Table 11, columns 1 and 2). In general, the time window during which a specific action should take place (e.g. taking of a blood sample) is rather narrow early in the study and becomes wider later in the study, because the influence of time-since-treatment on the exact value of the variable to be obtained (e.g. haemoglobin concentration, drug concentration) is expected to be less later in the study than earlier (e.g. for a pharmacokinetic analysis, the time window during which blood samples are to be taken during the first hours after drug administration is measured in minutes, while that spanning, for example, days 1–7 after treatment is in hours).

It is unlikely that all patients will undergo an evaluation within the protocol-defined time windows. This is in particular true for the follow-up visits during the outpatient phase of the trial.

To ensure an unbiased data analysis, time windows need to be defined (prior to the data becoming available for analysis) that determine into which analysis the data from a specific patient who has a follow-up visit outside the protocol-scheduled time windows is to be slotted. These time windows need to cover the whole follow-up period in a contiguous way, such that the data from all subjects can be included in the analysis, no matter how different the actual visit date is from the specific protocol-scheduled visit date.

The Informal Consultation recommended the use of the time windows and slotting rules described in Table 11.

Table 11
**Slotting of actual follow-up visit time-points for efficacy data analysis**

| **Protocol plan** | | **Study conduct** | **Data analysis** |
|---|---|---|---|
| **Follow-up visit (ideal follow-up time)** | **Protocol-defined acceptable time window around the ideal follow-up time[a]** | **Actual time follow-up data are obtained for subjects** | **Efficacy analysis time-point to which the data will be assigned** |
| End of treatment | Within 2 days, latest 14 days after treatment | 1–30 days after end of treatment[b] | End of treatment |
| 3 months | At 3 months ± 1 week after end of treatment | 2–4 months after end of treatment | 3 months |
| 6 months | At 6 months ± 2 weeks after end of treatment | 5–9 months after end of treatment | 6 months |
| 12 months | At 12 months ± 4 weeks after end of treatment | 10–16 months after end of treatment | 12 months |
| 18 months | At 18 months ± 4 weeks after end of treatment | 17–21 months after end of treatment | 18 months (test-of-cure visit) |
| 24 months | At 24 months ± 4 weeks after end of treatment | ≥ 22 months after end of treatment | Included in 18 months follow-up assessment (test-of-cure visit) for evaluation of efficacy of drugs<br>Note: If the objective of the analysis is to determine the time of relapse after treatment and the follow-up data available support this, these data may be analysed separately |

[a] These time windows are provided for illustration only, and are not specifically recommended by the Informal Consultation.

[b] In all cases, the time period begins on the first day of the first month and lasts until the last day of the last month.

Given the considerations described in section 4 in particular, the fact that the participants in the Informal Consultation deemed that a follow-up time-point of 12 months could not be recommended at the present time, the window for the 18-months ToC visit was chosen to be asymmetrical (i.e. 17–21 months) to avoid the recommendation for an 18 months follow-up time-point becoming in reality a 17- or 16- or 15-months efficacy time-point. As a consequence and/or to avoid slotting people with very disparate follow-up time-points, the windows around the 6- and 12-months follow-up time-points have different widths on the two sides of the preferred time-point.

For a clinical trial evaluating the efficacy of a drug, which includes patients who were not diagnosed as relapsed before the 18-month visit and who were not evaluated for ToC until well after 18 months (up to the last time after treatment for which the protocol requires attempts to be made to localize the patients), a differentiation between patients having their ToC visit at 18 months or at 24 months after treatment is in general not necessary.

For data analyses designed to determine the fraction of patients relapsing at 12 versus 18 versus 24 months of treatment, patients having their ToC visit e.g. 17 months and 23 months after treatment should be analysed separately, provided that the follow-up data available justify such an analysis.

An additional analysis with different data slotting may be indicated, depending on the objectives of the analysis.

## 6.3 Handling of missing efficacy data

Prior experience (see Table A1.14) shows that the number of patients with missing follow-up data for at least one planned follow-up time-point, is large, even in trials with active follow-up, and that this number increases with time since treatment.

To ensure comparability of data from trials conducted by different investigators/sponsors, the way in which patients for whom data concerning the ToC visit are missing are included in the efficacy analysis should be uniform. Table 12 summarizes the recommendations for categorizing for quantification of efficacy among patients without a ToC evaluation and who have not reached a protocol-defined end-point (e.g. death, probable relapse, relapse) before the ToC visit.

Table 12
**Inclusion into the efficacy analysis of patients who have not reached a protocol-defined end-point and for whom there are no data for the 18 months test-of-cure visit**

Table 12

| Results of interim follow-up evaluation | | | | Test-of-cure visit | Inclusion into efficacy analysis as |
|---|---|---|---|---|---|
| **3 or 6 months follow-up (≤ 9 months after treatment)** | **Additional follow-ups (≤ 9 months after treatment)** | **12 months follow-up (10–16 months after treatment)** | **Additional follow-ups (10–16 months after treatment)** | **18 months (17–21 months after treatment or ≥ 17 months)[a]** | |
| Missing | NA | Missing | NA | Missing | Not included |
| Missing | NA | UE | Missing | Missing | Unknown |
| Missing | NA | UE | UE | Missing | Unknown |
| Missing | NA | UE | FE | Missing | PC |
| Missing | NA | FE | NA | Missing | PC |
| UE | UE or Missing | Missing | NA | Missing | Unknown |
| UE | UE or Missing | UE | Missing | Missing | Unknown |
| UE | UE or Missing | UE | UE | Missing | Unknown |
| UE | UE or Missing | UE | FE | Missing | PC |
| UE | UE or Missing | FE | NA | Missing | PC |
| UE | UE | Missing | NA | Missing | Unknown |
| UE | UE | UE | Missing | Missing | Unknown |
| UE | UE | UE | UE | Missing | Unknown |
| UE | UE | UE | FE | Missing | PC |
| UE | UE | FE | NA | Missing | PC |
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| UE | FE | UE | FE | Missing | PC |
| UE | FE | FE | NA | Missing | PC |
| FE | NA | Missing | NA | Missing | Unknown |
| FE | NA | UE | Missing | Missing | Unknown |
| FE | NA | UE | UE | Missing | Unknown |
| FE | NA | UE | FE | Missing | PC |
| FE | NA | FE | NA | Missing | PC |

FE, Favourable evolution; NA, Not applicable; PC, Probable cure; UE, Uncertain evolution.
[a] See last two paragraphs in Table 11.

## 6.4 Efficacy variables

### *6.4.1 Nomenclature*

A uniform use of different terms to refer to measures of the efficacy of a drug (as proposed in Table 13) in combination with uniform calculation of efficacy measures (as proposed in Table 14) will facilitate comparison between data reported from different trials and treatment protocols.

Table 13
**Nomenclature for different measures of drug efficacy**

| Variable | Drug effect referred to |
|---|---|
| **Treatment fatality rate** | Quantifies the lack of efficacy of the drug in terms of deaths attributable either to lack of curative effect or to toxicity. Subjects who died from unknown causes are included based on the considerations underlying their inclusion in the treatment failure rate. |
| **Treatment failure rate** | Quantifies failure of the drug to result in cure of patient independent of whether this failure is attributable to lack of efficacy or to toxicity of the drug. Since, in particular for regulatory purposes, the efficacy reported should be overstated, treatment failure should also include those patients who died from unknown causes (and for whom it is thus not certain that they did not die because of lack of efficacy or toxicity of the drug under evaluation) and who discontinued owing to toxicity of the drug. |
| **Relapse rate** | Quantifies the lack of efficacy of the drug via the number of patients who did not have detectable levels of trypanosomes at the end of treatment, but were diagnosed as relapsed or probably relapsed. Depending on the objectives of the analysis, the calculation of the overall relapse rate (i.e. including 'relapse' and 'probable relapse') may be complemented by the calculation of 'parasitologically confirmed relapse rate' and 'probable relapse rate'. |
| **Cure rate** | Quantifies the efficacy of the drug via the number of patients who were classified as 'cure' or 'probable cure'.<br>Depending on the objectives of the analysis, the calculation of the overall cure rate (i.e. including 'cure' and 'probable cure') may be complemented by the calculation of 'parasitologically confirmed cure rate' and 'probable cure rate'. |
| **Response rate** | Quantifies the efficacy of the drug via the number of patients who were found to be parasite-free at the end of treatment. This measure is of particular importance for the first studies of new treatment or new combinations of established treatments. |
| **Non-response rate** | Quantifies the lack of efficacy of the drug in terms of its ability to clear trypanosomes from body fluids by the end of treatment. |

### *6.4.2 Calculation of efficacy*

A series of efficacy variables to be calculated was defined on the basis of requirements for regulatory submissions for new drugs and comparative interpretation of results of different studies. Which of the efficacy variables defined below should be chosen as the primary efficacy variable will differ between different studies, depending on the objectives of the study (e.g. pivotal versus dose-finding study).

The patients to be included in the nominator and denominator for the different measures of efficacy are provided in Table 14. Definition and criteria for the different response categories in the nominator are those provided in section 5 and Figure 1.

Each of these variables can be calculated using different denominators, i.e. for different analysis populations as well as for different time-points after treatment (e.g. treatment failure rate at EoT or at a specified time-point during interim follow-up), depending on the objectives of the analysis.

Table 14
**Description of efficacy variables**

| Variable | Nominator | Denominator |
|---|---|---|
| Treatment fatality rate | Sum of patients who:<br>▪ died during treatment (likely) due to HAT<br>▪ died during treatment (likely) due to treatment related adverse events<br>▪ died during follow-up (likely) due to HAT<br>▪ died during follow-up (likely) due to treatment related adverse events<br>▪ died from unknown causes | Safety analysis set |
| Treatment failure rate | Sum of patients who:<br>▪ died during treatment due to HAT<br>▪ died during treatment due to treatment-related adverse events<br>▪ died during follow-up (likely) due to HAT<br>▪ died during follow-up (likely) due to treatment-related adverse events<br>▪ died during treatment or follow-up due to unknown causes<br>▪ were non-responders at EoT visit<br>▪ relapsed<br>▪ probably relapsed<br>▪ were discontinued from study treatment due to treatment-related adverse events (and not cured) | Full analysis set |
| *Relapse rate* | | |
| Overall relapse rate | Sum of patients who:<br>▪ relapsed<br>▪ probably relapsed | Full analysis set<br>Per-protocol set<br>Modified per-protocol set |
| Parasitologically confirmed relapse rate | Patients who:<br>relapsed | Full analysis set<br>Per-protocol set<br>Modified per-protocol set |
| Probable relapse rate | Patients who:<br>▪ probably relapsed | Full analysis set<br>Per-protocol set<br>Modified per-protocol set |
| *Cure rate* | | |
| Parasitologically confirmed cure rate | Sum of patients who:<br>▪ were cured | Full analysis set<br>Per-protocol set<br>Modified per-protocol set |
| Probable cure rate | Sum of patients who:<br>▪ were probably cured | Full analysis set<br>Per-protocol set<br>Modified per-protocol set |
| Response rate | Patients:<br>▪ who were responders at EoT visit | Safety analysis set<br>Full analysis set |
| Non-response rate | Patients:<br>▪ who were non-responders at EoT visit | Safety analysis set<br>Full analysis set |

EoT: end-of-treatment; HAT: human African trypanosomiasis

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# Appendix

## Briefing document

## for the

## Informal Consultation on Clinical Trials for Trypanosomiasis

**Geneva, 9–10 September 2004**

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# 1. Introduction

The conduct of clinical trials for the evaluation of new treatment modalities for human African trypanosomiasis (HAT) faces a number of challenges that are rarely, if ever, encountered in this combination in other diseases. A large number of these challenges are related to the fact that both the disease and the populations it affects are neglected. Thus, there is no background of generally accepted—and ubiquitously feasible—diagnostic and treatment standards that usually form the basis for planning and conducting clinical evaluation of new treatment modalities for a disease.

Over the past years, interest in evaluation and development of new treatment modalities has increased and it thus appears timely to try and establish a common approach that will facilitate collaboration in the evaluation of new treatment modalities and/or facilitate comparison of data obtained by different groups.

This meeting has been convened for the very different organizations interested in the development of new treatment modalities for HAT to get together and discuss past experiences and current approaches to patient care and evaluation of new treatment modalities to see whether common approaches are desirable—and feasible.

This document provides a short overview of major issues that the medical and scientific community face as a basis for the discussions during the meeting. While the data summarized deal with *Trypanosoma brucei gambiense* HAT, similar considerations apply to *T. b. rhodesiense* HAT.

# 2. Diagnostic criteria

## 2.1 Criteria for diagnosis of first- and second-stage African trypanosomiasis caused by *T. b. gambiense*

### *2.1.1 Diagnosis*

The diagnosis of *T. b. gambiense* HAT follows a three-step pathway: screening, diagnostic confirmation and staging. For the purpose of screening of the population at risk for *T. b. gambiense*, detection of trypanosome-specific antibodies in blood by the card agglutination test for trypanosomiasis (CATT/*T. b. gambiense*), is currently used in most endemic areas. Diagnostic confirmation in CATT-positives or clinical suspects then relies on the finding of trypanosomes through microscopical examination of body fluids.

Criteria for diagnostic confirmation of HAT are uniform when trypanosomes are detected in the blood, lymph nodes or CSF (parasitological confirmation). Because of the limited sensitivity of the parasitological techniques (see section 2.3.2) and the sometimes low parasitaemia, failure to demonstrate parasites does not necessarily exclude infection.

Some control programmes/investigators/nongovernmental-organization treatment programmes, therefore, take into account additional criteria such as the end-dilution titre of CATT performed on serum dilutions and/or white blood cells (WBCs) in the CSF and/or clinical signs (examples in Table A1.1).

Table A1.1
**Control-programme case definition of HAT caused by *T. b. gambiense*, in 2001**

| Country | Case definition |
|---|---|
| Benin, Cameroon, Guinea, Central African Republic, Chad, Togo | Trypanosomes detected |
| Democratic Republic of the Congo | Trypanosomes detected OR<br>No trypanosomes but clinical signs and > 5 WBC/µl CSF |
| Gabon | Trypanosomes detected OR<br>No trypanosomes but CATT + ≥ 1/2 |
| Congo | Trypanosomes detected OR<br>No trypanosomes but CATT + ≥ 1/4 |
| Uganda | Trypanosomes detected OR<br>No trypanosomes but CATT + ≥ 1/4 and > 20 WBC/µl CSF |
| Sudan[a] | Trypanosomes detected OR<br>No trypanosomes but CATT + ≥ 1/4 and > 20 WBC/µl CSF OR<br>No trypanosomes but CATT + ≥ 1/16 and prevalence of HAT among tested population > 2% |
| Angola, Equatorial Guinea | Trypanosomes detected OR<br>No trypanosomes but CATT + ≥ 1/8 |

CATT: card agglutination test for trypanosomiasis; CSF: cerebrospinal fluid; HAT: human African trypanosomiasis; WBC: white blood cells

Source: Simarro et al. (2003).

[a] Programme carried out by Médecins Sans Frontières, Switzerland, and reported in Chappuis et al. (2004).

There is no universal algorithm for the diagnosis of HAT in cases that remain parasitologically unconfirmed. Criteria to define a CATT-positive case as a patient with HAT, to perform a lumbar puncture, and whether or not to follow up CATT-positives, differ substantially. These criteria are influenced by:

- Different positive predictive values of CATT depending on HAT prevalence;
- Workload;
- Political situation, climate/season, transport and communication links, project means and resources.

### *2.1.2 Staging*

Staging of the disease is a key step that allows classification of the patient into the first (haemolymphatic) stage or the second (meningoencephalitic) stage of the disease and is based on CSF examination. The WHO criteria (WHO, 1998) to diagnose second-stage infection in parasitologically confirmed cases are based on the presence in CSF of:

- Trypanosomes; and/or
- An elevated WBC count of > 5 WBC/µl; and/or
- An elevated protein content (> 370 mg/l using the dye-binding assay).

It has been reported that protein determination from CSF has been abandoned almost completely due to the need for sophisticated material, instability of reagents, and limited additional information obtained from the analysis (Lejon et al., 2003).

Although a cut-off of 5 cells/µl CSF is most widely applied, other cut-off criteria for the cell count are in use. Moreover, some organizations/countries apply different cut-offs for cell count in the CSF for parasitologically unconfirmed CATT-positives who are considered to be patients, than for individuals in whom infection was parasitologically confirmed in blood or lymph-node aspirate (Table A1.2).

Table A1.2
**Criteria for second-stage HAT caused by *T. b. gambiense***

| Investigators | Criteria | Reference |
|---|---|---|
| *Clinical trials/Treatment programmes* | | |
| MSF Sudan (2001 or later) | Trypanosomes in CSF AND/OR<br>> 5 WBC/µl CSF AND trypanosomes in blood or lymph OR<br>≥ 20 WBC/µl CSF AND CATT titration ≥ 1/4 AND absence of trypanosomes in blood or lymph | Chappuis F, personal communication |
| Malteser (June 2002 or later)<br>Burri et al. (2000)<br>Milord et al. (1992) | Trypanosomes in CSF AND/OR<br>> 5 WBC/µl CSF | Milord et al. (1992); Burri et al. (2000); Franco et al. (2004) |
| WHO/TDR (2000) | Trypanosomes in CSF AND/OR<br>> 20 WBC/µl of CSF | WHO 2000 |
| Marion Merrel Dow, Eflornithine registration studies (1980s) | Trypanosomes in the CSF, AND/OR<br>elevated CSF WBC count, elevated CSF protein concentration, presence of detectable IgM end-dilution titers in CSF AND/OR characteristic signs and symptoms of CSF involvement | WHO/TDR and Aventis (2003) |
| *WHO/National control programmes* | | |
| WHO Expert Committee | Trypanosomes in CSF AND/OR<br>> 5 WBC/µl CSF OR > 37 mg of protein/100 ml (dye-binding protein assay) | WHO (1998) |
| Democratic Republic of the Congo, Congo, Sudan | Trypanosomes in the CSF AND/OR<br>> 5 WBC/µl CSF | Schmid et al. (2005) |
| Equatorial Guinea | Trypanosomes in the CSF AND/OR<br>≥ 10 WBC/µl CSF | Schmid et al. (2005) |
| Angola, Côte d'Ivoire | Trypanosomes in the CSF AND/OR<br>≥ 20 WBC/µl CSF | Josenando (1994); Doua et al. (1996); Schmid et al. (2005) |

CATT: card agglutination test for trypanosomiasis; CSF: cerebrospinal fluid; HAT: human African trypanosomiasis; TDR: UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases; WBC: white blood cells

Some data on the percentage of second-stage patients who are categorized as such without detection of parasites are summarized in Table A1.3.

Table A1.3
**Patients with HAT categorized as second-stage in the absence of parasites**

| Site/study | No. of second-stage patients | Trypanosome-negative in all tests (staging based on CATT and WBC) | | Trypanosome-negative CSF (staging based on WBC and signs and symptoms) | | References |
|---|---|---|---|---|---|---|
| | | N | (%) | N | % | |
| MSF, Omugo, Uganda | 1785 | 112 | (6.3) | Data not available | | Priotto G, personal communication |
| MSF, Ibba, Sudan | 3135 | 118 | (3.6) | Data not available | | Priotto G, personal communication |
| MSF, Kiri, South Sudan | 1376 | 104 | (7.6%) | Data not available | | Chappuis F, personal communication |
| Impamel I | 500 | 103 | (20.6%) | 451 | (90.7%) | Burri C, personal communication |
| Impamel II | 2571 | 406 | (15.8%) | 1006 | (39.1%) | Schmid C, personal communication |

CATT: card agglutination test for trypanosomiasis; HAT: human African trypanosomiasis; MSF: Médecins Sans Frontières; WBC: white blood cells

## Questions for discussion

1 Why are different criteria used for staging of *T. b. gambiense* HAT in the absence of trypanosomes in the CSF?

2 What is the impact of different criteria for staging in the absence of trypanosomes in the CSF in terms of comparability of the results of different clinical studies?

3 Can one/should one agree on common criteria for staging for clinical trial inclusion criteria?

### 2.2 Criteria for diagnosing relapse

#### *2.2.1 Diagnosis of relapse in first-stage HAT*

The criteria for relapse after treatment for first-stage HAT and assessment of whether a relapsed patient is first-stage or second-stage are identical or similar to those for the diagnosis of the disease, depending on the criteria for initial diagnosis and staging used.

### *2.2.2 Diagnosis of relapse (or re-infection) in second-stage HAT*

Relapse is uniformly and unambiguously diagnosed by the presence of trypanosomes in blood, lymph, and/or CSF in patients in whom no trypanosomes were detectable immediately after the EoT. The data to distinguish between relapse and re-infection are usually not available and thus the number of patients diagnosed as relapsed possibly includes patients who have been re-infected. The probability of patients diagnosed as relapsed being re-infections will depend on the time after treatment and the infection rates in the areas where they live and is likely relatively small.

If trypanosomes are not detected, diagnosis of relapse is based on the absolute number of WBCs and/or an increase in WBCs in the CSF with or without neurological symptoms of the disease. Criteria differ somewhat between different investigators/organizations conducting disease control or clinical studies. Table A1.4 provides an overview of criteria used.

Table A1.4
**Criteria for diagnosing relapse of patients with second-stage HAT**

| Investigator | Criteria | References |
|---|---|---|
| Malteser (2002) | Trypanosomes in CSF, lymph node or blood AND/OR<br>WBC/µl CSF clearly higher than at previous examination | Franco et al. (2004) |
| MSF (Uganda, Sudan) | Trypanosomes in blood, lymph or CSF AND/OR<br>> 20 WBC/µl of CSF at 24 months AND/OR<br>> 20 WBC/µl of CSF at < 24 months and higher than the previous two counts AND/OR<br>> 20 WBC/µl of CSF at < 24 months and neurological signs | Priotto (2004); Chappuis (2004) |
| Burri et al. (IMPAMEL I) | Trypanosomes in lymph, blood or CSF AND/OR<br>> 50 WBC/µl CSF and twice that at previous evaluation OR<br>20–49 WBC/µl CSF and symptoms of relapse (somnolence, long-lasting headache, recurrent fever) | Burri et al. (2000); WHO (2000) |
| Schmid et al. (IMPAMEL II) | Relapse<br>Trypanosomes in blood, lymph or CSF<br>Suspected relapse<br>> 50 WBC/µl CSF and twice that at previous evaluation OR<br>WBC count is 6–49 cells/µl and clear symptoms attributed to relapse (somnolence, long-lasting headache, recurrent fever) | Schmid et al. (2005) |

| Investigator | Criteria | References |
|---|---|---|
| Burri et al.<br>(DB289) | Relapse<br>Trypanosomes in blood, lymph or CSF<br>Suspected relapse<br>> 50 WBC/µl CSF and twice that at previous evaluation OR<br>WBC count is 6–49 cells/µl and clear symptoms attributed to relapse (somnolence, long-lasting headache, recurrent fever) | Burri C, Schmid C, personal communication |
| Milord et al. (1992) | Trypanosomes in the blood or CSF AND/OR<br>≥ 50 WBC/µl CSF | Milord et al. (1992) |
| Marion Merrel Dow, Eflornithine registration studies (1980/1990s) | Trypanosomes in CSF AND/OR<br>Obvious increase in CSF WBC count, CSF WBC above normal limits | WHO/TDR and Aventis (2003) |
| Bisser S et al. | Trypanosomes in the blood, lymph of CSF AND/OR<br>> 20 WBC/µl CSF and at least twice that at previous evaluation AND/OR<br>>20 WBC/µl CSF at 24 months after treatment | Bisser S et al. (in press) |
| Pépin J, Milord F | Trypanosomes in the blood or CSF AND/OR<br>Cell count higher than previous count and ≥ 50 WBC/µl CSF AND/OR<br>Cell count higher than previous count, 20–49 WBC/µl and recurrence of symptoms | Pépin & Milord (1994) |
| Lourie EM | Definition of significant increase/decrease in cell count:<br>Initial count, 0–4: significant increase is to > 10<br>Initial count, 5–20: significant increase is to more than double the initial count<br>Initial count, > 20: significant reduction is to less than square root of the original | Lourie (1942) |

CSF: cerebrospinal fluid; HAT: human African trypanosomiasis; WBC: white blood cell

### *2.2.3 Diagnosis of 'suspected relapse'*

Some investigators have introduced a category of 'suspected relapse'. This includes patients who show clinical symptoms of relapse, but in whom relapse could not be confirmed parasitologically. These patients may then undergo a pre-defined series of follow-up investigations before being diagnosed as confirmed relapses (see section 2.5).

### *2.2.4 Diagnosis of cure*

Patients are classified as 'cured' if there is no evidence of relapse at the pre-determined time-point, in general, currently 24 months after treatment based on recommendations of a 1986 WHO Expert Committee on Epidemiology and control of African trypanosomiasis (WHO Expert Committee on Epidemiology and Control of African Trypanosomiasis, 1986). For the purposes of ongoing updates on efficacy evaluation in a patient group, patients who have not been diagnosed as relapsed at a certain time after treatment are classified as 'cured at *x* months' by some investigators.

## Questions for discussion

4 Why are different non-parasitological criteria used for determining relapse in the absence of trypanosomes in the CSF?

5 What is the impact of different criteria for relapse in the absence of trypanosomes in the CSF in terms of comparability/assessment of the results of different clinical trials?

6 What criteria for relapse should be applied on parasitologically unconfirmed CATT positive individuals who are considered as patients?

7 Can one/should one agree on common non-parasitological criteria?

### 2.3 Methodological issues

#### *2.3.1 Serological assessment*

The CATT is the only serological test applicable in field situations for screening of the population at risk for *T. b. gambiense* infection. As the sensitivity of the CATT depends on the variable antigen type of the circulating trypanosomes, performance may differ according to the geographical origin of the patients (Table A1.5). Sensitivity of the screening test on whole blood ranges between 83% and 100%, specificity between 78% and 97%. Specificity increases when CATT is performed on serum dilutions, a principle that is at the basis of determining subgroups of CATT-positives at risk, and of including the CATT end-dilution titre into the definition of a case. Two groups have reported that it has indeed been demonstrated that individuals with a CATT end-dilution titre of $\geq 1:16$ are at a high risk of having trypanosomiasis (Simarro et al., 1999; Chappuis et al., 2004).

It has been reported that CATT cannot be used for follow-up since about half of the patients remain positive for up to 24–36 months after treatment (Paquet et al., 1992) and some patients even stay positive for up to 5 years after successful treatment (Miézan et al., 2002).

#### *2.3.2 Parasite detection*

As all parasitological techniques rely on detection of the trypanosome, they should have 100% specificity, assuming sufficient training of the personnel.

The measured sensitivity of parasite detection tests depends on:

- The detection limit of the respective test (Table A1.6);
- Distribution of the parasitaemia; and
- The choice of a gold standard.

Table A1.5

**Performance of CATT for diagnosis of HAT in different regions**

| Test version | Sensitivity (%) | Specificity (%) | Country | Author |
|---|---|---|---|---|
| CATT-whole blood | ND | 96.1 | South Africa | Bafort et al. (1986) |
| CATT-whole blood | 98.9–100.0 | 78.0–88.0 | Democratic Republic of the Congo | Pépin (1986) |
| CATT-whole blood | 83.3 | 96.8 | Congo | Noireau et al. (1988) |
| CATT-whole blood | 91.7 | 92.5 | Côte d'Ivoire | Jamonneau (2001) |
| CATT-whole blood | 98.2 | 94.0 | Central African Republic | Truc et al. (2002) |
| CATT-whole blood | 100.0 | 96.7 | Côte d'Ivoire | |
| CATT-whole blood | 100.0 | 90.3 | Côte d'Ivoire | Magnus et al. (2002) |
| CATT-whole blood | 90.4 | 96.5 | Uganda, Equatorial Guinea, Democratic Republic of the Congo | |
| CATT-whole blood | 100.0 | 94.3 | Cameroon | Penchenier et al. (2003) |
| CATT-serum 1:4 | 92.3 | 100.0 | Caucasian, Democratic Republic of the Congo, Upper Volta, Côte d'Ivoire | Magnus et al. (1978) |
| CATT-serum 1:5 | 96.8 | 94.3 | Congo | Noireau et al. (1988) |
| CATT-serum 1:10 | 91.6 | 99.0 | Congo | |
| CATT-serum | 99.8 | ND | Côte d'Ivoire | Miézan et al. (2002) |

CATT: card agglutination test for trypanosomiasis; HAT: human African trypanosomiasis; ND: not done

Table A1.6:

**Detection limits reported for parasitological methods for the detection of HAT**

| Technique[a] | Theoretical value (trypanosomes/ml) | Reported detection limit (trypanosomes/ml) |
|---|---|---|
| Lymph-node aspirate | 50–100 | |
| Fresh blood examination | 100 | 6000–10000 |
| Thick blood film | 40 | 600–5000 |
| Haematocrit[b] centrifugation (Woo, 1971) | 15 | 500–600 |
| mAECT (Lumsden et al., 1979) | 5 | 15–100 |
| QBC (Bailey & Smith, 1992) | 15 | 15–300 (Ancelle et al., 1997)[c] |

Source: Adapted from Arbyn (1993)

HAT: human African trypanosomiasis ; mAECT: mini-anion exchange centrifugation technique; QBC: quantitative buffy coat

[a] References are for particular techniques developed by the given author

[b] Erythrocyte volume fraction

[c] Most recently reported detection limit

The theoretical detection limit of techniques for the detection of parasites in CSF depends on the volume of CSF that is examined. Ideally, one trypanosome per volume examined should be detectable by the concentration techniques. By direct examination of CSF, a maximum of 5 µl CSF is examined, corresponding to a detection limit of 200 trypanosomes/ml.

None of the existing trypanosome detection techniques is 100% sensitive. Evaluation of parasitological methods is hampered by the absence of a real gold standard and the reported diagnostic sensitivities differ according to the chosen reference tests (Table A1.7, Table A1.8).

Table A1.7
**Diagnostic sensitivity of parasitological techniques for the detection of HAT in blood or lymph**

| Author | Lymph-node aspirate (%) | Fresh blood examination (%) | Thick blood film (%) | Haematocrit centrifugation (%) | mAECT (%) | QBC (%) |
|---|---|---|---|---|---|---|
| Duvallet et al. (1979) | 52 | ND | ND | 86 | ND | ND |
| Henry et al. (1981) | 33 | 54 | ND | 82 | ND | ND |
| Lumsden et al. (1981)[a] | ND | ND | 31<br>63 | 46<br>73 | 88<br>100 | ND |
| Dukes et al. (1984) | ND | 14 | 57 | 29 | 43 | ND |
| Molisho (1992) | 25 | ND | 41 | ND | 93 | ND |
| Bailey & Smith (1992) | 69 | ND | 93 | ND | ND | 100 |
| Truc et al. (1994) | 63 | ND | 100 | 44 | 80 | 93 |
| Miézan et al. (1994) | 59 | 22 | 35 | 48 | 85 | ND |
| Truc et al. (1998) | ND | ND | ND | ND | 100 | 85 |

mAECT: mini-anion exchange centrifugation technique; HAT: human African trypanosomiasis; ND: not done; QBC: quantitative buffy coat

[a] Two different methods to estimate sensitivity were used: (1) six patients known to be infected were repeatedly examined; (2) 35 mAECT-positives were examined by other techniques.

Table A1.8
**Sensitivity of parasitological techniques for the detection of HAT in CSF**

| Author | Direct examination (%) | Simple centrifugation (%) | Double centrifugation (%) | Modified simple centrifugation (%) |
|---|---|---|---|---|
| Cattand et al. (1988)[a] | ND | 45 | 100 | ND |
| Miézan et al. (1994)[b] | 19 | 41 | 69 | ND |
| Miézan et al. (2000)[a] | ND | ND | 82 | 97 |

CSF: cerebrospinal fluid; HAT: human African trypanosomiasis; ND: not done

[a] Gold standard is finding of trypanosomes in CSF

[b] Gold standard is finding of trypanosomes in any body fluid

For diagnosis, any of the parasitological techniques is valid (although higher sensitivity can be expected using concentration techniques). From the above tables it is, however, clear, that for assessment of the disease stage and cure in clinical trials, concentration techniques are more appropriate.

Owing to the interplay between parasite antigenic variation and the patient's immune system, the numbers of parasites in the blood of the patient may vary considerably. As a consequence, the parasite concentration may remain under the detection limit at certain moments, while at other moments parasites can be easily detected. In case of high suspicion of infection or relapse, it is advisable to repeat parasitology on different occasions if trypanosomes cannot be detected immediately.

In the reality of field use, the sensitivity and specificity of the parasite detection depends not only on the method used (e.g. single or double centrifugation) but also on the level of training of the personnel (e.g. centrifugation technique, handling of samples, differentiation of trypanosomes from microfilarial worms) and the quality of the equipment (e.g. microscopes, other laboratory equipment).

## Questions for discussion

8 Can we/do we need to agree on common methods for inclusion criteria and efficacy parameter assessment for the purpose of clinical trials?

### 2.4 Potential surrogate markers for cure/relapse

#### *2.4.1 Currently available data on surrogate end-points*

A few alternative markers for follow-up of patients are suggested in the literature, mainly IgM and trypanosome-specific antibody in CSF.

Already in 1967, Mattern had observed that relapses are always accompanied or even announced by an increase of IgM in the serum as well as in the CSF (Mattern, 1967). According to Knobloch et al. (1984), "efficacy of treatment is indicated by a decrease of IgM in CSF, CSF total protein, and probably CSF trypanosome specific antibody". Greenwood & Whittle (1973) state that "a fall in CSF IgM follows successful treatment, but it may be weeks or months before normal levels are obtained".

A more detailed illustration of the evolution of IgM in CSF is shown in Whittle et al., 1977, according to whom the measurement of IgM in the CSF is of great help in diagnosis and management. Although they observed that IgM levels decrease slowly to normal by 12 months after treatment, a high level at that time or a rise after treatment are described as helpful in diagnosing relapsed patients. Moreover, they state that at any time after treatment, absence of IgM in a patient with a high CSF protein and raised cell count should cast doubt on the diagnosis of relapse.

It has been described that antibodies in the CSF drop quickly after successful treatment and the decrease of trypanosome-specific antibody concentrations in CSF has been proposed as an interesting parameter for definite cure (Roffi et al., 1979; Knobloch et al., 1984; Smith et al., 1989). This was confirmed more recently in a large group of patients infected with *T. b. gambiense* in Côte d'Ivoire (Miézan et al., 2002). Relapses in this group were characterized by the presence of trypanosome-specific antibodies.

From the same data, it has been reported (Cross & Jaffar, 2003) that in multivariate analysis, the presence of antibody in CSF measured at discharge was associated significantly with any relapse. Protein in CSF above the medial level of 36 mg/l was associated with relapse within 12 months of discharge, as was the presence of antibody in the CSF. At 12 months, a WBC count of > 50 WBC/µl CSF and antibody end-dilution titre of ≥ 2 were associated independently with relapse beyond 12 months.

Thus, although literature on surrogate markers for follow-up is scarce, promising alternative parameters have been suggested for assessment of cure or relapse, but have not found their way to the field. This might be owing to the lack of field-adapted tests.

The recent development of some field-adapted tests for assessment of disease stage and cure could change this situation.

- Based on the occurrence of a strong predominant intrathecal IgM synthesis in second-stage *T. b. gambiense* patients (Lejon et al., 2003), an experimental agglutination test for detection of IgM in the CSF, LATEX/IgM, was developed (Lejon et al., 2002). The reagent consists of anti-human IgM monoclonal antibodies covalently coupled to stained latex particles. Reagent and CSF are mixed on a card and rotated for 5 minutes. The presence of IgM is revealed by macroscopic agglutination. When testing serial dilutions of the CSF, an end-titre (highest dilution factor still giving a positive reaction) is obtained, which gives an estimate of the concentration of IgM in the CSF.
- LATEX/*T. b. gambiense* is an antibody-detecting agglutination test (Büscher et al., 1999). The reagent consists of semi-purified variable antigens of *T. b. gambiense* covalently coupled to latex particles in suspension. Although the test was developed for use on serum or blood, it can be applied on CSF. The reagent is mixed with the CSF on a card and rotated. The presence of trypanosome-specific antibodies is revealed by macroscopic agglutination.

Both tests remain to be validated on a large scale.

Other recent developments include:

- A dot–blot method to detect anti-neurofilament and anti-galactocerebroside antibodies in the CSF of patients with trypanosomiasis shows promising initial results for staging of the disease, but remains to be further evaluated (Courtioux et al., 2003). No data about the usefulness of these types of antibodies for follow-up are available yet.
- Patients with second-stage *T. b. gambiense* human African trypanosomiasis show a disruption of the 24-hour sleep–wake distribution with the abnormal occurrence of sleep-onset rapid eye movement (SOREM). This can be recorded by polysomnography, which could represent a non-invasive method to detect parasite invasion in the brain (Buguet et al., 2004).
- Different PCR assays exist, but none of them are yet validated for diagnostic purposes (Schares & Mehlitz, 1996; Kabiri et al., 1999; Jamonneau et al., 2001; Welburn et al., 2001; Radwanska et al., 2002). In principle, PCR can be used for any patient's sample that may contain trypanosome DNA, like whole blood or the buffy coat, lymph-node fluid or CSF, but results are not always unequivocal.

### *2.4.2 Validation of surrogate end-points*

In view of the variable current criteria for relapse, the high number of cases lost-to-follow-up, the lumbar punctures needed during follow-up and the long follow-up period, additional studies are required on:

- Methodological improvement of cell count and trypanosome detection, and definition of cell count-related relapse criteria based on scientific evidence;
- New follow-up criteria;
- The combination of new and existing follow-up parameters.

Table A1.9 summarizes some studies and trials (ongoing or planned for the near future) involving analysis of CSF samples obtained during patient follow-up in order to detect relapses after treatment via potential surrogate markers of relapse.

Table A1.9
**Studies and trials involving laboratory analysis of follow-up samples of CSF**

| Activity | Investigator | Country | Analyses | Status |
|---|---|---|---|---|
| Retrospective study on 400 patients | Institute of Tropical Medicine | Democratic Republic of the Congo | CSF IL-10, CSF IgM, CSF-specific antibody, CSF protein, etc | Analyses started |
| Prospective study on 500 patients | Institute of Tropical Medicine | Democratic Republic of the Congo | CSF IL-10, CSF IgM, CSF-specific antibody, CSF protein, etc | Start of study in September 2004 |
| DB-289 trial | Swiss Tropical Institute | Democratic Republic of the Congo | CSF IgM | Ongoing |
| Clinical trial of eflornithine-nifurtimox | Médecins Sans Frontières/ Epicentre | Congo | CSF IgM | Ongoing |

CSF: cerebrospinal fluid; IgM: immunoglobulin; IL: interleukin

## Questions for discussion

9 Are the ongoing and planned studies sufficient for the validation of these new tests? If not, what types of additional field trials are needed?

10 What is the present and future role of these new tests for staging of HAT and diagnosis of relapse in clinical trials?

## 2.5 Patient evaluation for relapse/cure in clinical trials

Patients are evaluated for treatment efficacy in general at the EoT and at different times after treatment. Most frequently the targeted follow-up time-points are 6, 12, 18 and 24 months after treatment. Since the direct or surrogate markers for relapse and decision on rescue treatment include CSF parameters (trypanosomes, WBCs, potentially IgM or

trypanosome specific antibodies), a lumbar puncture has to be performed at follow-up. For many patients, fear of the lumbar puncture for different reasons is a significant deterrent for attending follow-up investigations, in particular if they are feeling well.

If trypanosomes are detected in the blood, lymph or CSF, diagnosis of relapse is unambiguous. Further follow-up of patients is required for the patients' health, but from a clinical trial point of view, the end-point has been reached.

For patients who are suspected to have relapsed based on WBC counts, immunodiagnostic parameters or signs and symptoms of disease, different algorithms for follow-up may be used by different investigators and these have implications for the time that relapse is diagnosed and consequently for the time the rescue treatment is initiated. All methods/algorithms for follow-up and diagnosis of relapse include lumbar punctures.

Algorithms for follow-up of patients in clinical trials include (Burri C, personal communication; TDR protocol, in preparation):

- Suspicion of relapse based on WBC counts:
    - If there is at any follow-up examination an increase of the WBC count in the CSF of greater than 10 cells/µl, a test repetition (including microscopic examinations of lymph-node aspirate and blood) will be done 1 month later.
    - If at test repetition no trypanosomes are detected and there is no further increase of the CSF WBC count, the patient will be asked to return for additional testing on a 3-monthly basis.
    - If at any follow-up examination no trypanosomes are detected and there is a further increase of the CSF WBC count that cannot be explained by another likely diagnosis, the patient will be considered as a suspected treatment failure and treated as per the planned rescue treatment in the protocol.
    - If parasites are detected at any follow-up examination, the patient will be considered as a confirmed treatment failure.

Immunodiagnostics may be used during follow-up for the identification of patients who require particular attention. The basic algorithm is the same as used for follow-up of patients suspected to have relapsed based on CSF WBC count (Burri C. personal communication).

- Suspicion of relapsed based on immunodiagnostic test results:
    - If there is at least a fourfold increase in the CSF-end-dilution titre of LATEX/IgM test compared with the post-treatment value or the preceding follow-up value, a test repetition (including microscopic examinations of blood and lymph-node aspirate) will be done 1 month later.
    - If at test repetition no trypanosomes are detected and there is no further increase in the end-dilution titre, the patient will be asked to return for additional testing on a 3-monthly basis.
    - If at test repetition no trypanosomes are detected but there is a further increase of the end-dilution titre of the test, the patient will be considered as a suspected treatment failure and treated as per the planned rescue treatment in the protocol.

— If parasites are detected at any follow-up examination the patient will be considered as a confirmed treatment failure.

**Questions for discussion**

11 Can we agree on a common definition of who is suspected to have relapsed and who should undergo intense follow-up investigations?

12 What follow-up investigations should be performed on patients suspected to have relapsed? Can we / should we agree on a common algorithm?

13 Can we minimize the number of lumbar punctures during follow-up without jeopardizing patient safety?

## 3. HAT-specific challenges for the clinical evaluation of a new treatment modality

The conduct of clinical studies in patients with HAT faces several difficulties which are rarely encountered simultaneously in clinical trials for other tropical diseases, and never in non-tropical diseases of the developed countries. These include:

- The areas in which the disease is endemic, which are characterized by, for example, remoteness, extreme poverty and lack of, for example, transport and health care infrastructure;
- Lack of availability of sites suitably equipped and staffed for clinical trials;
- The usually very low endemicity even in identified disease foci, the slow disease development and the totally unspecific signs and symptoms of the disease which require a very large number of patients to be screened to identify patients;
- Elimination of the patient base within a reasonable distance from the site within a few years of the initiation of a new treatment site as a consequence of the successful treatment of the infected population in the vicinity of the site;
- A long follow-up period until efficacy can be ascertained (24 months after treatment according to currently accepted standards);
- A high loss to follow-up due to a combination of:
    - lack of transport infrastructure for the patients;
    - extreme poverty;
    - high mobility of the population even in areas without civil unrest;
    - high probability of political instability/civil unrest in some areas;
    - the long treatment follow-up required; and
    - patients' fear of the lumbar puncture at follow-up, especially among patients who do not experience signs and symptoms of the disease.

This section summarizes some of the data needed for discussion on how best evaluate the efficacy and safety of a new treatment modality under these conditions.

## 3.1 Requirements for build-up of clinical trial sites

In general, two independently conducted clinical studies, both powered for suitable statistical significance, are regarded as necessary to establish the efficacy and safety of a new treatment and, if applicable, its superiority or non-inferiority relative to the standard of care. For treatments with cure rate of ≥ 90% or a relapse rate of ≤ 10%, each of these studies needs to enrol sufficient patients to obtain several hundred patients who can be evaluated for drug efficacy.

Currently there are very few sites equipped and staffed to conduct clinical trials according to good clinical practice (GCP) for HAT. The implementation of a new clinical trial centre for trypanosomiasis includes significant improvement of the facility. This includes usually construction or structural improvement for the laboratory and patient accommodation and most often electrical installations. Often the equipment for the routine examinations is old and outdated, and all equipment linked to the study must be added. The laboratory staff needs to be re-trained in basic parasitological techniques, and to be introduced to all other methods (e.g. biochemistry, haematology). The nursing and laboratory staff has to undergo introduction to the principles of clinical trials including GCP training. The medical staff very often needs additional training in the observation and classification of symptoms and signs.

Mobile teams (i.e. a vehicle with a team of about eight people, which circulates in the foci identified) are critical for the conduct of a clinical trial, since only the use of active case search will normally yield sufficient patient numbers. Most national plans foresee the activity of mobile teams for active case search, but very often those structures do not exist or are only partially functional. Existing mobile teams must be appropriately trained and equipped with a lot of effort before the initiation of a trial.

It must be understood that the staff in rural centres is generally very limited and very often there is no physician, which implies further challenges for the conduct of clinical trials: first, it may be difficult to find additional staff to relocate and work under the given circumstances; and second, all developments need to be made in a sustainable way, to allow a durable improvement in diagnostic and treatment capacity.

Consequently, the conduct of two independent pivotal trials for the evaluation of the safety and efficacy of a new treatment modality for HAT is a major challenge and may not actually be feasible.

## 3.2 Identification of patients for clinical trials of new treatment modalities

The clinical trial-initiated and/or accelerated screening and treatment activities will lead to efficient detection and treatment of patients and thus to the elimination of the ‘patient reservoir for the trial’. Therefore, the same site can rarely be used for larger studies for more than 1 to 2 years in a row.

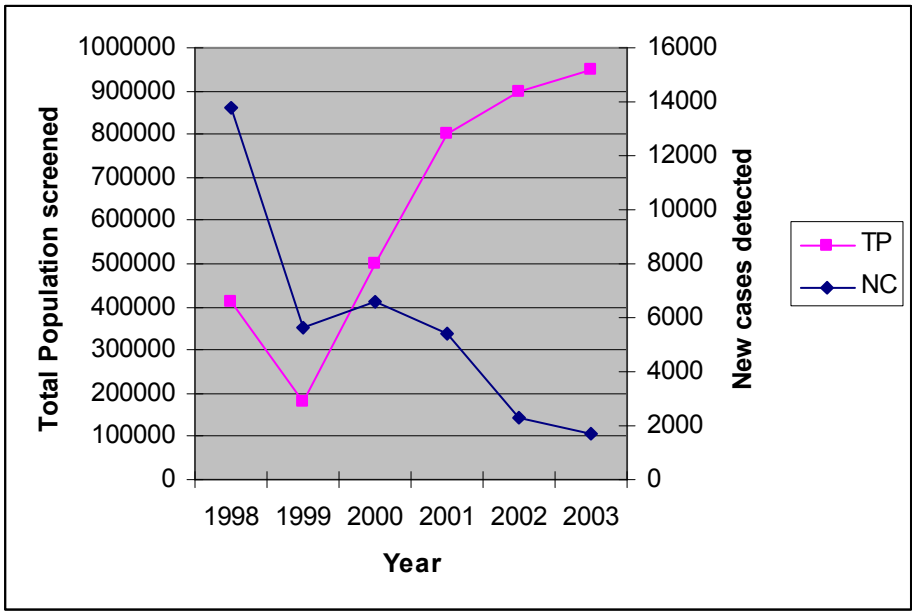
Table A1.10 and Figure A1.1 demonstrate that there has been a decrease by a factor of 30 in the numbers of detected cases within 6 years as a consequence of active case detection and treatment in the North Equatorial province of the Democratic Republic of the Congo.

Table A1.10
**Screening and case detection for HAT in a control programme in an endemic zone in the Democratic Republic of the Congo**

| Screening and case detection | 1997 | 2003 |
|---|---|---|
| Population screened | 286 919 | 949 747 |
| New cases (n) detected | 14 764 | 1 597 |
| New cases (%) of people screened | 5.14 | 0.17 |

HAT: human African trypanosomiasis
Source: N'Siesi (2004)

Figure A1.1
**Population screened and new cases of HAT detected between 1998 and 2003 in North Equatorial Province of the Democratic Republic of the Congo**




HAT: human African trypanosomiasis; TP: Total population screened, NC: new cases detected
Source: N'Siesi (2004)

Even in newly identified disease foci, the endemicity of HAT is usually very low. This, together with the slow disease development and the totally unspecific symptoms and signs of HAT, in particular in its first stage, requires screening of a very large number of patients for enrolment to clinical trials.

Table A1.11
**Number of patients screened and included in a trial of drugs to combat first-stage HAT**

| No. of patients screened and included | Viana (Angola) | | Maluku (Democratic Republic of the Congo) | | Vanga (Democratic Republic of the Congo) | |
|---|---|---|---|---|---|---|
| Total No. of persons screened | 10 451 | | 31 491 | | 12 253 | |
| Total No. of new cases identified (%) | 62 | (0.59%) | 298 | (0.95%) | 65 | (0.53%) |
| New cases, first stage | 6 | | 115 | | 31 | |
| Cases included (%) | 4 | (0.04%) | 28 | (0.09%) | 15 | (0.12%) |
| Cases excluded owing to | 5[a] | | 88[a] | | 16 | |
| Age | 3 | | 27 | | 10 | |
| Weight | — | | 14 | | 3 | |
| Abnormal ECG | 2 | | 11 | | — | |
| Other reasons | 0 | | 36 | | 3 | |

Source: Burri C, personal communication

ECG: electrocardiogram; HAT: human African trypanosomiasis

[a] Not complete agreement because additional patients were included from passive reporting to centre

## 3.3 Time of relapse in recent disease control treatment programmes and clinical studies

The currently accepted standard follow-up time for efficacy for patients treated for late stage HAT is 24 months after treatment. Eflornithine was approved in the USA based on data from 282 treated patients, including 49 patients with ≥ 2 years of follow-up (all cured), 80 with 12–23.5 months of follow-up and 64 with 3–11.5 months of follow-up. Eflornithine was approved in France based on data from 556 treated patients, including 114 with ≥ 2 years of follow-up and 103 with 12–23.5 months of follow-up (Marrion Merrel Dow, 1988).

Under the conditions under which clinical trials in HAT patients have to be conducted, obtaining follow-up for a significant fraction of the patients requires 'active' follow-up (i.e. mobile teams of appropriately trained personnel travelling out to find and evaluate the patients). The longer the follow-up time, the more effort needs to be invested into the follow-up and the lower the probability of obtaining complete follow-up data (for details see section 3.4). Thus, it is worthwhile to review the data available on the time of detected relapse to evaluate whether 24 months after treatment is the only time or the best time at which to assess the efficacy of a new treatment modality.

### *3.3.1 Relapse of patients with first-stage HAT*

*Relapses after suramin*

Suramin is the drug of choice for first-stage *T. b. rhodesiense* infection. Cure rates of over 95% have been reported (Apted FIC, 1980), but also high failure rates in the range of 25–35% (Neujean, 1950; Veeken et al., 1989). Initially, suramin was also used against *T. b .gambiense,* with a very high efficacy (Harding, 1945), but today suramin is no longer used for *T. b. gambiense* infection except in combination with pentamidine. The combination of suramin plus pentamidine was considered to be less toxic than monotherapy and to cause fewer relapses (Williamson, 1970). However, Pepin & Khonde (1996) question the usefulness of the combination of two drugs which are known to penetrate very poorly into the central nervous system. They reported that in vitro a drug exposure of 1 μg/ml for 24 hours is sufficient to inactivate the bloodstream forms.

It is not known whether suramin-resistant *T. b. gambiense* or *T. b. rhodesiense* strains exist today. Observed relapses could be attributed to second-stage infections, which were not diagnosed as such. Since it is known that suramin penetrates very poorly into the central nervous system, this drug is not expected to cure a second-stage infection at the doses used in humans.

*Relapses after pentamidine*

The reported relapse rates after a course of five injections were all in the same range of approximately 7% (Jonchère, 1951; Dutertre & Labusquiere, 1966). Such relapses could be explained by second-stage infections, which were misdiagnosed as first-stage, rather than by pentamidine-refractory HAT/pentamidine-resistant trypanosomes, which have not been described so far in the field. Neujean & Evens (1958) reported that 16% of pentamidine-treated patients relapsed, but could subsequently be cured with melarsoprol.

Small amounts of pentamidine corresponding to 0.5–0.8% of the plasma concentrations were found in the CSF of all patients after the last dose of a 10-day treatment course. It has been reported (Bronner et al., 1991; Bronner, 1994) that pentamidine generally persisted in CSF for at least 30 days. In vitro, concentrations as low as 10 ng/ml have a trypanocidal effect during prolonged exposure (Miézan et al., 1994b). On the basis of these results, the use of pentamidine for so-called early–late-stage patients with a WBC count of up to 20 cells/$mm^3$ instead of 5 cells/$mm^3$ was proposed (Doua et al., 1996). In a preliminary study, a small number of patients was treated accordingly to the new definition. Although the rate of relapse after pentamidine treatment was slightly higher than after treatment with melarsoprol (6% versus 3.7%), the new definition is now in use as a standard in Angola; and publication of the results is pending.

Table A1.12 summarizes the data on time of relapse of patients diagnosed as early-stage and treated with pentamidine in the Médecins Sans Frontières treatment programme in Arua, Uganda, with active follow-up. The overall relapse rate, calculated based on the number of patients discharged alive was 7.1%. Among the 26 patients who were diagnosed as relapsed, 25 patients were staged after relapse as stage 2.

Table A1.12
**Time of at which relapse was diagnosed in MSF treatment programme for first-stage HAT in Arua, Uganda, 1995–2000**

| Programme | Treated (treatment) | Discharged alive | Treated ≥ 24 months before data cut-off | Patients diagnosed as relapsed, by months after treatment (% of total relapsed) | | | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Total | ≤ 6 | ≤ 11.5 | 12–17.5 | 18–23.5 | ≥ 24 | |
| Active follow-up[a] | | | | | | | | | | |
| Control | 677 (pentamidine) | 675 | 675 | 48[a] | 5 (10) | 9 (19) | 16 (33) | 8 (16.7) | 15 (31) | Priotto G, personal communication (11 August 2004) |

HAT: human African trypanosomiasis; MSF: Médecins Sans Frontières

[a] Includes two patients who died during follow-up independent of cause of death

### *3.3.2 Relapse of patients with second- stage HAT*

Table A.1.13 summarizes data on the time of diagnosis of relapse from field treatment protocols and clinical studies. The relapses at different times after treatment are quantified as percentage of total number of patients diagnosed as relapsed.

Studies/programmes with active follow-up are listed separated from those with passive follow-up. For active follow-up, patients were actively sought out for follow-up examinations. For passive follow-up, no specific efforts were undertaken to reach patients for follow-up, so that under-diagnosis of early relapses as well as diagnosis of total relapses are less accurate than in studies with active follow-up. Consequently, studies with active follow-up provide a more accurate database for assessment of the time of relapse after treatment.

Table A1.13
**Time at which relapse was diagnosed in control programmes and clinical studies on second-stage HAT**

| Programme | Treated (treatment) | Discharged alive[c] | Treated ≥ 24 months before data cut-off | Patients diagnosed as relapsed, by months after treatment (% of total relapsed)[a,b] | | | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Total | ≤ 6 | ≤ 11.5 | 12–17.5 | 18–23.5 | ≥ 24 | |
| *Active follow-up*[d] | | | | | | | | | | |
| Control/clinical studies | 1557[e] (different, see footnote e) | 1530 | 1530 | 406 | 131 (33) | 252 (62) | 93 (25) | 26 (6) | 35 (9) | Priotto (2004) |
| | 1422 new cases (melarsoprol) | 1363 | 1363 | 398 | 69 (17) | 224 (56) | 96 (24) | 35 (9) | 43 (11) | |
| Control | 1402 (melarsoprol) | 1402 | 900 | 26[f] | 0–9 months: 19 (73) | | 9–18 months: 5 (19) | 18–30 months: 2 (8) | | Chappuis (2004) |
| Control | 587 (eflornithine) | 573[g] | 111[g] | 25 | 10 (40) | | 22 (88) | 3 (12) | | Franco et al. (2004) |
| Clinical study (Impamel I)[h] | 500 (melarsoprol) | 483 | 483 | 25 | 8 (32) | 12 (48) | 6 (24) | 5 (20) | 2 (8) | Schmid et al. (2004) |
| *Passive follow-up*[h] | | | | | | | | | | |
| Treatment[i] | Not available (melarsoprol) | 812 | 812 | 32 | 16 (50) | 16 (50) | 8 (25) | 7 (21.9) | 1 (3.1) | Cross & Jaffar (2003) |
| Field study (Impamel II) | 2571 (melarsoprol) | 2423 | 2423 | 179 | 115 (64) | 162 (91) | 10 (6) | 3 (2) | 4 (2) | Schmid (2004) |

HAT: human African trypanosomiasis

[a] Includes patients who relapsed ≤ 6 months after treatment

[b] Relapsed patients includes patients who died during follow-up, irrespective of the cause of death.

[c] Discharged alive, excluding deaths shortly after discharge d Studies/programmes with active follow-up are separated from those with passive follow-up. For active follow-up, patients were actively sought out for follow-up. For passive follow-up, no specific efforts were undertaken to reach patients for follow-up, so that underdiagnosis of early relapses and total relapses are less accurate than in studies with active follow-up.

[e] Patients included received melarsoprol treatment (after prior relapse), eflornithine for 7 days, eflornithine for 14 days, nifurtimox-melarsoprol combination, nifurtimox-eflornithine, melarsoprol-eflornithine.

[f] Relapses among 900 patients who completed all scheduled follow-up investigations at 6, 12 and 24 months.

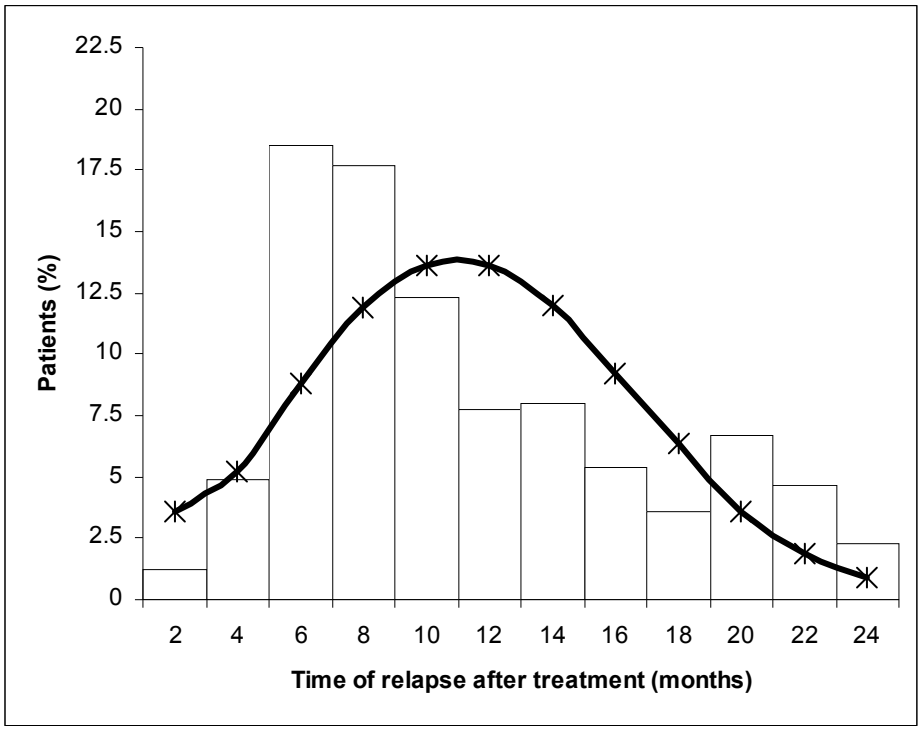
[g] Patients who completed treatment and were discharged. At the time of data cut-off for this analysis, 503 patients had been treated at ≥ 6 months, 423 patients at ≥ 12 months and 111 patients at ≥ 24 months before data cut-off. Relapse rates were 10/503 in the 6 months after treatment, 21/423 6–12 months after treatment and 14/111 during 13–24 months after treatment.

[h] The high number of patients diagnosed as relapsed in the second year after treatment is attributed to underdiagnosis during the first year and efforts to follow up all patients in the second year who had not been seen during the first year after treatment.

[i] All relapses reported for the period of 12–17.5 months were diagnosed at around 12 months after treatment.

Data on time of relapse determined by HATSENTINEL[4] from three different sites in the Democratic Republic of the Congo are shown in Figure A1.2. These hospitals do some active follow-up. However, the majority of patients were diagnosed as relapsed when they came to the clinic on their own initiative.

Figure A1.2
**Time of relapse detection among 371 patients diagnosed as relapsed within the HATSENTINEL in East Kasai Province of the Democratic Republic of the Congo**




Source: Data generated by HATSENTINEL

For interpretation of the data in Table A1.13, it needs to be kept in mind that for all those data sets where the number of patients treated is larger than the number of patients with at least 24 months passed since treatment, there is some bias towards relapses earlier on after treatment, since the number of patients who completed the specified period of time after treatment (and thus contributed to relapses at that time-point) is the smaller, the later after treatment it is. This bias is increased when the percentage of patients seen for follow-up decreases with increasing time between treatment and follow-up.

[4] HATSENTINEL is a network to monitor treatment failure and drugs resistance for HAT. It was implemented in July 2002 and continues as of October 2006. It was developed by the Centers for Disease Control and Prevention (CDC), Division of Parasitic Diseases, National Center for Zoonotic, Vector-borne and Enteric Diseases, Coordinating Center for Infectious Diseases. CDC is a WHO Collaborating Centre for Surveillance of Human African Trypanosomiasis, Treatment Failure, and Drug Resistance. The HATSENTINEL sentinel surveillance network consists of nine sites to date; seven sites are located in areas endemic for *T. b. gambiense* (Angola, Democratic Republic of the Congo, Sudan) and two sites are in areas endemic for *T. b. rhodesiense* (Uganda, United Republic of Tanzania)

Table A1.14 provides more information on follow-up data available for the data in Table A1.13.

On the other hand, in the IMPAMEL I study (Schmid et al., 2004), specific follow-up efforts during the second year after treatment for patients who had not presented for follow-up during the first year, may have resulted in relapses that occurred during the first year after treatment being diagnosed and recorded only during the second year. This would result in a bias towards relapses later on after treatment: nine patients diagnosed as relapsed 12–24 months after treatment had had either no previous follow-up examination or the previous follow-up examination was more than 10 months ago.

Because of the environment in which HAT is endemic and the nature of the disease, it is not possible to make assumptions regarding the fate of patients not seen for follow-up—patients may be cured and thus do not see the necessity to invest what may be considerable resources for them into a visit to the treatment centre and to have to undergo lumbar puncture or may have relapsed and be unable (physically and/or financially) to visit the treatment centre.

Within these limitations, the data in Table A1.13 do suggest that between 70% and 90% of relapses occur within 18 months after treatment, and between 40% and 90% of relapses occur already within 12 months after treatment.

### 3.4 Follow-up of HAT patients

Experience over the past 20 years has shown that under the conditions in which treatment/control programmes and clinical studies for HAT are conducted, follow-up of patients as per protocol (in clinical studies and nongovernmental organization-conducted treatment programmes, typically 6, 12, and 24 months after treatment; in other control programmes, ideally at least once at 24 months after treatment) is always incomplete.

Table A1.14 summarizes published and unpublished data on follow-up of first- and second-stage HAT patients.

In the tables, the number of patients seen for follow-up relative to the number of patients expected to be seen for follow-up was quantified, i.e. all patients who were discharged alive, whose follow-up was due at the time-point specified and who had not relapsed or died before the time-point specified.

Table A1.14
**Follow-up rates in control programmes and clinical studies on HAT**

| Programme | Treated (treatment) | Country | Follow-up | Patients seen for follow-up/patients expected for follow-up (patients seen as % of patients expected)[a] | | | | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | ≤ 11.5 months | 12–17.5 months | 18–23.5 months | ≥ 24 months | |
| *Active follow-up*[b] | | | | | | | | |
| Control | 638 (pentamidine) | Sudan (Yei) | Active | 389/509 (76) | 218/354 (26) | Not available | 19/70 (26) | Franco et al. (2004) |
| Treatment programme | 732 (pentamidine) | Uganda (Arua) | Active | 626/727 (86) | 10–18 months 576/716 (80) | > 18 months 523/692 (76) | | Priotto G, personal communication (Epicentre database, introduction to briefing document, 11 August 2004) |
| Control | 760 (pentamidine) | Sudan (Kiri) | Active | 3–9 months 500/760 (66%) | 9–18 months 364/748 (49%) | 18–30 months 216/740 (29%) | | Chappuis F, personal communication, 16 August 2004 |
| Control/clinical studies | 1422 (melarsoprol, new cases) | Uganda (Arua) | Active | 0–9 months 1149/1345 (85) | 10–18 months 919/1175 (78) | > 18 months 748/1048 (71) | | Priotto, G (Epicentre), 8 July 2004 |
| Control/clinical studies | 825 (melarsoprol, eflornithine, nifurtimox) | Uganda (Arua) | Active | 0–9 months 628/756 (83) | 10–18 months 463/585 (79) | > 18 months 390/493 (79) | | Priotto G, personal communication (Epicentre database, introduction to briefing document, 11 August 2004) |
| Control | 1402 (melarsoprol) | Sudan (Kiri) | Active | 3–9 months 830/1288 (64.4) | 9–18 months 549/1173 (46.8) | 18–30 months 267/900 (29.7) | | Chappuis (2004) |

| Programme | Treated (treatment) | Country | Follow-up | Patients seen for follow-up/patients expected for follow-up (patients seen as % of patients expected)[a] | | | | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | ≤ 11.5 months | 12–17.5 months | 18–23.5 months | ≥ 24 months | |
| Control | 587 (eflornithine) | Sudan (Yei) | Active | 405/503 (80.5) | 284/413 (68.8) | Not applicable | 45/100 (45.0) | Franco et al. (2004) |
| Clinical study (Impamel I) | 500 (melarsoprol) | Angola | Active | 413/483 (85.5) | 12–23.5 months 301/463 (65.0) | | 60/426 (14.1) | Schmid et al. (2004) |
| *Mixed active–passive follow-up* | | | | | | | | |
| Field study (Impamel II)[b] | 2571 (melarsoprol) | See below | Mixed | 1089/2423 (45) | 264/2245 (12) | 55/2235 (2.5) | 29/2233 (1.3) | Schmid (2004) |
| | 651 | Sudan – IMC | Active? | 338/627 (54) | 33/507 (7) | 8/492 (2) | 0 | |
| | 23 | Equatorial Guinea | Active | 15/21 (71) | 9/18 (50) | 10/18 (56) | 4/18 (22) | |
| | 504 | Sudan (Kiri) | Active | 304/480 (63) | 123/457 (27) | 15/456 (3) | 20/456 (4) | |
| | 30 | Central African Republic | Unknown | 28/29 (97) | 26/28 (93) | 0 | 0 | |
| | 27 | Côte d'Ivoire | Unknown | 25/25 (100) | 6/24 (25) | 0 | 0 | |
| | 547 | Angola | Passive | 58/515 (11) | 0 | 0 | 0 | |
| | 561 | Democratic Republic of the Congo | Passive | 191/516 (37) | 62/509 (12) | 21/500 (4) | 4/500 (1) | |
| | 228 | Congo | Passive | 126/210 (60) | 2/192 (1) | 0 | 1/192 (1) | |

| Programme | Treated (treatment) | Country | Follow-up | Patients seen for follow-up/patients expected for follow-up (patients seen as % of patients expected)[a] | | | | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | ≤ 11.5 months | 12–17.5 months | 18–23.5 months | ≥ 24 months | |
| *Passive follow-up*[b] | | | | | | | | |
| Treatment | Not available, discharged 812 (melarsoprol) | Côte d'Ivoire | Passive | 429/812 (52.8) | 345/812 (42.5) | 276/812 (34.0) | 279/812 (34.4) | Cross & Jaffar (2003) |

HAT: human African trypanosomiasis; IMC: International Medical Corps

[a] Excludes patients who died during treatment or shortly after treatment.

[b] Studies and programmes with active follow-up are separated from those with passive follow-up. For active follow-up, patients were actively sought for follow-up examinations.

The data in Table A1.14 show that even in nongovernmental-organization treatment programmes or clinical studies with active patient follow-up, follow-up rates drop significantly with time since treatment.

### 3.5 Calculation of efficacy

Efficacy in studies of infectious diseases is usually quantified as the number of patients that fulfil the pre-defined criteria for 'treated successfully' or 'treated unsuccessfully' as a percentage of:

(a) all patients who received at least one dose of study medication ('intent-to-treat' population);

(b) all patients who received treatment as prescribed by the protocol ('per-protocol' population).

Data on whether or not a patient fulfils the criteria for 'treated successfully' or 'treated unsuccessfully' are expected to be available for the vast majority of patients treated in the 'intent-to-treat' population and the 'per-protocol' population (population that can be evaluated for treatment efficacy).

In most studies of infectious disease, the time of follow-up required until the determination of successful or unsuccessful treatment is only a few weeks. This contributes to making it possible to have the required data for the complete or nearly complete 'intent-to-treat' and 'per-protocol' populations.

The data in Table A1.14 show that for HAT patients it is not possible to achieve close to 100% follow-up at any time after treatment and that follow-up rates drop with time after treatment. Thus, the choice of timing for determination of the nominator and of an appropriate denominator for accurate absolute and relative quantification of efficacy of a new treatment modality for HAT is a major issue.

## Questions for discussion

14 The data in Table A1.13 show that the majority of patients who relapse within 24 months after treatment do so within the first 12 to 18 months. The data in Table A1.14 show that even with active follow-up it is in most cases impossible to obtain 24 months follow-up on > 80% of patients.

(a) Is final efficacy assessment at 12 or 18 months sufficient to assess the value of a new treatment modality in a pivotal trial? Can a final efficacy assessment at 12 or 18 months improve the reliability of the efficacy quantitation because data are based on a higher percentage of patients evaluated?

(b) What additional value for the assessment of efficacy of a new treatment modality is provided by 24 months follow-up data?

(c) For dose finding or proof of concept studies, a 24 months follow-up period is not realistic. What types of efficacy data provide an adequate basis for deciding on a pivotal study and the dose to be evaluated in it?

15 If a significant percentage of patients are lost to follow-up, this creates uncertainty as to how cure and relapse rates should be calculated. This situation is further complicated by the fact that most of the patients with follow-up data at one time-point are not available for follow-up at another time-point, and that some patients may not consent to a CSF sample being taken (the pre-requisite for parasitological confirmation of cure/relapse) at each or any follow-up time-point. This makes comparison of cure or relapse rates at different times after treatment difficult, since (i) the numerator is based on different patient populations; and (ii) the denominator, if calculated as the number of patients with follow-up data, differs between follow-up time-points. Furthermore, comparison between treatment arms of the study is complicated if the percentage of patients with follow-up data differs substantially between treatment arms.

16 Denominator for relapse and cure rates: is the number of patients discharged alive a better (more consistent) denominator for cure or relapse rates than the traditional 'number of patients with follow-up data'?

17 **Primary efficacy variable**:

(a) Is the parasitologically confirmed treatment failure rate, calculated as follows, a suitable primary efficacy variable:

> Number of patients with trypanosomes detected in the blood, lymph-node aspirate or CSF at any follow-up examination (and without distinction between relapse and re-infection) as a percentage of the number of patients who received at least one dose of treatment and were discharged alive from the hospital.

(b) Should patients treated for second-stage HAT and for whom no trypanosomes are detectable in blood, lymph or CSF, but who are suspected to have relapsed based on an increase in the CSF WBC count of > 10 cells/µl be included in the calculation of treatment failure rate? Or should those patients be quantitated separately to obtain a secondary efficacy variable?

(c) Should patients without parasites in blood, lymph or CSF and without a CSF WBC count of > 10 WBC/µl CSF (e.g. patients who refuse to undergo a lumbar puncture) who are suspected to have relapsed based on signs and symptoms of the disease be included among patients in the calculation of the treatment failure rate? Or should 'clinically suspected relapses' be quantified separately to obtain a secondary efficacy variable?

(d) Should the primary efficacy variable instead be the 'failure rate' calculated as the sum of the number of patients who:

— had parasites in the blood, lymph or CSF at the EoT;

— relapsed (i.e. with demonstrated presence of parasites in the CSF) before or at the chosen efficacy end-point time);

— suspected to have relapsed based on WBC count > 10 WBC/µl CSF and/or signs and symptoms of HAT;

— died during treatment or follow-up with death due to HAT;

— died during treatment or follow-up due to drug-related or disease-related adverse events; or who

— died during follow-up with unknown cause of death,

as a percentage of all patients who received at least one dose of treatment?

(e) Are the following suitable **secondary efficacy variables**:

— Relapse rate or failure rate in the 'per-protocol' population, calculated as the number of patients in whom treatment failed/or who relapsed before or at the chosen efficacy end-point time (as defined for the primary efficacy parameter) or as the number of patients who failed treatment (see above for primary efficacy variable) as a percentage of the number of patients discharged alive from the hospital who received treatment as planned in the protocol?

— Response rate at EoT: the percentage of patients released from the hospital with no evidence of parasites in the CSF, blood or lymph among all patients treated (intent-to-treat) and all patients treated as per protocol?

— Fatality rate at the EoT: the percentage of patients who died due to HAT before the scheduled EoT among all patients treated?

(f) Can 'time to relapse' or 'time to failure' statistics provide additional or better assessment of efficacy than calculation of rates?

18 A database to support the conclusion that a new drug is adequately safe and effective has nowadays to include data from at least two independently conducted clinical studies, both showing similar results. The exact requirements tend to be indication-specific.

(a) Given the small number of sites available at any one time to conduct clinical studies in HAT patients according to GCP, the large number of patients that need to be screened and the fact that each site has only a limited 'lifetime':

— Is it feasible to conduct two independent trials, both powered for statistical significance (estimated at around 500–600 patients)?

— Is it feasible to conduct one trial powered for statistical significance and a second smaller 'supportive' study?

— Can one appropriately-powered trial provide sufficient evidence of the safety and efficacy of a new treatment modality in HAT?

— How can the limiting factors (resources, site personnel recruitment and training, study conduct, active follow-up) for site build-up be reduced?

# References


Ancelle T et al. (1997). Détection des trypanosomes dans le sang par la technique du "quantitative buffy coat"(QBC): évaluation expérimentale. *Médecine Tropicale,* 57:245–248.

Apted FIC (1980). Present status of chemotherapy and chemoprophylaxis of human trypanosomiasis in the eastern hemisphere. *Pharmacology and Therapeutics*, 11:391–413.

Arbyn M (1993). Etat actuel de la connaissance sur les méthodes de dépistage et de diagnostic de la maladie du sommeil (deuxième édition). *Instituut voor Tropische Geneeskunde, Antwerpen, Belgium*, 1–103.

Bafort JM, Schutte CHJ, Gathiram V (1986). Specificity of the Testryp CATT card agglutination test in a non-sleeping-sickness area of Africa. *South African Medical Journal,* 69:541–542.

Bailey JW, Smith DH (1992). The use of the acridine orange QBC technique in the diagnosis of African trypanosomiasis. *Transaction of the Royal Society of Tropical Medicine and Hygiene,* 86:630.

Bisser S et al. Equivalence trial on melarsoprol and nifurtimox mono- and combination therapy for treatment of second-stage *Trypanosoma brucei gambiense* sleeping sickness. *Journal of Infectious Diseases*. In press.

Bronner U (1994). Pharmacokinetics of pentamidine. Focus on treatment of *Trypanosoma gambiense* sleeping sickness [PhD thesis]. Stockholm, Karolinska Institute.

Bronner U et al. (1991) Pentamidine concentrations in plasma, whole blood and cerebrospinal fluid during treatment of *Trypanosoma gambiense* infection in Côte d'Ivoire. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 85:608–611.

Buguet A et al. (2004). Les troubles du cycle veille-sommeil dans la trypanosomose humaine africaine [The polysomnographic syndrome in HAT]. *Premier Congrès International de Brazzaville sur la mouche tsé-tsé et les trypanosomoses*, *23–25 March 2004,* Brazzaville, Congo.

Burri C et al. (2000) Efficacy of new concise schedule for melarsoprol for treatment of sleeping sickness caused by *Trypanosoma brucei gambiense*: a randomized trial. *Lancet,* 355:1419–1425.

Büscher P et al. (1999). Improved latex agglutination test for detection of antibodies in serum and cerebrospinal fluid of *Trypanosoma brucei gambiense*-infected patients. *Acta Tropica*, 73:11–20.

Cattand P, Miézan BT, de Raadt P (1988) Human African trypanosomiasis: use of double centrifugation of cerebrospinal fluid to detect trypanosomes. *Bulletin of the World Health Organization,* 66:83–86.

Chappuis F (2004) Post treatment follow-up in Kiri, Kajo-Keji County, South Sudan. July 2004.

Chappuis F et al. (2004). Card agglutination test for trypanosomiasis (CATT) end-dilution titer and cerebrospinal fluid cell count as predictors of human African trypanosomiasis (*Trypanosoma brucei gambiense*) among serologically suspected individuals in southern Sudan. *The American Journal of Tropical Medicine and Hygiene*, 71:313–317.

Courtioux B et al. (2003). A dot-blotting method for the diagnosis of the CNS involvement in human African trypanosomiasis. *International Colloquium Cerebrospinal Fluid Analysis in Tropical Neurology, 26–29 November 2003*, Antwerp, Belgium.

Cross P, Jaffar S (2003). Report on the Trypanosome Cohort data (812 patients diagnosed with *T. b. gambiense* infection in Daloa between January 1983 and January 1992, submitted to the WHO/TDR Product Development Team, 18 July 2003.

Doua F et al. (1996). The efficacy of pentamidine in the treatment of early-late stage *Trypanosoma brucei gambiense* trypanosomiasis. *The American Journal of Tropical Medicine and Hygiene*, 55:586–588.

Dukes P et al. (1984). A field comparison of seven diagnostic techniques for human trypanosomiasis in the Luangwa Valley, Zambia. *Tropenmedizin und Parasitologie,* 35:141–147.

Dumas M, Girard PL (1978). Human African trypanosomiasis (sleeping sickness). In: Vinken PJ & Bruyn GW, eds. *Handbook of Clinical Neurology. Vol. 35: Infections of the nervous system. Part III*. Amsterdam, North-Holland Publishing Company, 67–83.

Dutertre J, Labusquiere R (1966). La thérapeutique de la trypanosomiase. *Médecine Tropicale (Marseille),* 26:342–356.

Duvallet G et al. (1979). Trypanosomiase humaine Africaine: centrifugation en tube capillaire. *Nouvelle Presse Médicale*, 8:214–215.

Franco JR, Abirigo J, Hughes S (2004). Results of DFMO treatment in Malteser Yei. March 2002–June 2004. Compiled 15 July 2004, for the briefing document for the Informal Consultation.

Greenwood BM, Whittle HC (1973). Cerebrospinal-fluid IgM in patients with sleeping-sickness. *Lancet,* 2:525–527.

Harding RD (1945). Late results of treatment of sleeping sickness in Sierra Leone by antrypol, tryparsamide, pentamidine and propamidine singly and in various combinations. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 3:99–124.

Henry MC et al. (1981). Evaluation du diagnostic sur le terrain de la trypanosomiase à *Trypanosoma brucei gambiense*. *Annales de la Société belge de médecine tropicale* 61:79–92.

Jamonneau V, Solano P, Cuny G (2001). [Use of molecular biology in the diagnosis of human African trypanosomiasis]. *Médecine Tropicale*, 61:347–354.

Jonchère H (1951). [Diamidine therapy of the lymphatic-blood phase of human trypanosomiasis in French Equatorial Africa]. *Bulletin de la Société de pathologie exotique et de ses filiales*, 44:603–625,

Josenando T (1994). Manual de regras para o controlo da tripanossomíase humana africana. Luanda, Angola, National Control Programme.

Kabiri M et al. (1999). Detection of *Trypanosoma brucei gambiense* in sleeping sickness suspects by PCR amplification of expression-site-associated genes 6 and 7. *Tropical Medicine and International Health*, 4:658–661.

Knobloch J et al. (1984). Evaluation of immunoassays for diagnosis and management of sleeping sickness in Liberia. *Tropenmedizin und Parasitologie*, 35:137–140.

Lejon V et al. (2002). IgM quantification in the cerebrospinal fluid of sleeping sickness patients by a latex card agglutination test. *Tropical Medicine and International Health,* 7:685–692.

Lejon V et al. (2003). Intrathecal immune response pattern for improved diagnosis of central nervous system involvement in trypanosomiasis. *The Journal of Infectious Diseases,* 187:1475–1483.

Lourie EM (1942). Treatment of sleeping sickness in Sierra Leone. *Tropical Medicine And Parasitology,* 36:113–131.

Lumsden WHR et al. (1979). *Trypanosoma brucei*: miniature anion-exchange centrifugation technique for detection of low parasitaemias: adaptation for field use. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 73:312–317.

Lumsden WHR et al. (1981). Field diagnosis of sleeping sickness in the Ivory Coast. I. Comparison of the miniature anion-exchange / centrifugation technique with other protozoological methods. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 75:242–250.

Magnus E, Vervoort T, Van Meirvenne N (1978). A card-agglutination test with stained trypanosomes (CATT) for the serological diagnosis of *T. b. gambiense* trypanosomiasis. *Annales de la Société belge de médecine tropicale*, 58:169–176.

Magnus E et al. (2002). Evaluation of an EDTA version of CATT/*T. b. gambiense* for serological screening of human blood samples. *Acta Tropica,* 81:7–12.

Marrion Merrel Dow (1988). New drug application for ornidyl injection to United States Food and Drug Administration, New Drug Application No. 19–879. Kansas, MO, United States of America.

Mattern P (1967). Etude du taux de gamma-globuline dans le liquide céphalo-rachidien. *Septième Conférence technique OCCGE (Mars),* 1053–1056.

Miézan TW et al. (1994a). Evaluation des techniques parasitologiques utilisées dans le diagnostic de la trypanosomose humaine à *Trypanosoma gambiense* en Côte d'Ivoire. *Bulletin de la Société de pathologie exotique et de ses filiales,* 87:101–104.

Miézan TW et al. (1994b). Long-term exposure of Trypanosoma brucei gambiense to pentamidine in vitro. Transactions of the Royal Society of Tropical Medicine and Hygiene, 88:332–333.

Miézan TW et al. (2000). Single centrifugation of cerebrospinal fluid in a sealed pasteur pipette for simple, rapid and sensitive detection of trypanosomes. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 94:293.

Miézan TW et al. (2002). Trypanosomose humaine africaine en Côte d'Ivoire: caractéristiques biologiques après traitement. A propos de 812 cas traités dans le foyer de Daloa (Côte d'Ivoire). *Bulletin de la Société de pathologie exotique et de ses filiales,* 95:362–365.

Milord F et al. (1992). Efficacy and toxicity of eflornithine for treatment of *Trypanosoma brucei gambiense* sleeping sickness. *Lancet,* 340:652–655.

Molisho S (1992). Place des techniques sérologiques et parasitologiques dans le dépistage de la trypanosomiase à *Trypanosoma brucei gambiense* par les équipes mobiles au Zaïre. In: Habbema JDF, De Muynck A, eds. *Rapport final du séminaire de modélisation appliquée pour l'optimisation des prises de décisions et du suivi des programmes de contrôle de la maladie du sommeil*, *28–30 October 1992*. Daloa, Côte d'Ivoire, 67–72.

Neujean G (1950). Contribution à l'étude des liquides rachidiens et céphaliques dans la maladie du sommeil à *Trypanosoma gambiense*. *Annales de la Société Belge de Médecine Tropicale,* 30:1125–1387.

Neujean G, Evens F (1958). Diagnostic et traitement de la maladie de sommeil à *T. b gambiense*. Bilan de dix ans d'activité au centre de traitement de Léopoldville. *Académie Royale des Sciences Coloniales: Classes des Sciences Naturelles et Médicales; Mémoires in 8,* Vol. VII.

Noireau F et al. (1988). Serodiagnosis of sleeping sickness in the Republic of the Congo: comparison of indirect immunofluorescent antibody test and card agglutination test. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 82:237–240.

N'Siesi F-X (2004). Presentation at *Les deuxièmes journées scientifiques sur les trypanosomoses, 26–28 July 2004*, INRB, Kinshasa, Democratic Republic of the Congo.

Paquet C (1992). Persistence of antibodies to *Trypanosoma brucei gambiense* after treatment of human trypanosomiasis in Uganda. *Lancet*, 340:250.

Penchenier L et al. (2003). Evaluation of LATEX/*T. b. gambiense* for mass screening of *Trypanosoma brucei gambiense* sleeping sickness in Central Africa. *Acta Tropica* 85:31–37.

Pépin J (1986). Utilisation du Testryp CATT pour le dépistage de la trypanosomiase à Nioki, Zaïre. *Annales de la Société Belge de Médecine Tropicale* 66:213–224.

Pepin J, Khonde N (1996). Relapses following treatment of early-stage *Trypanosoma brucei gambiense* sleeping sickness with a combination of pentamidine and suramin. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 90:183–186.

Pépin J, Milord F (1994). The treatment of human African trypanosomiasis. *Advances in Parasitology* 33:2–49.

Priotto G (2004). Analysis of follow-up rates and time to relapse of patients treatment between 1995 and 2002 for HAT in Omuga, Arua District, Uganda. France, Médecins Sans Frontières. Compiled for this briefing document, 8 July 2004.

Radwanska M et al. (2002). The serum resistance-associated gene as a diagnostic tool for the detection of *Trypanosoma brucei rhodesiense*. *The American Journal of Tropical Medicine and Hygiene,* 67:684–690.

Roffi J, Dedet JP, Garre MT (1979). Diagnostic immuno-enzymatique de la trypanosomiase en phase nerveuse par mise en évidence d'anticorps spécifiques dans le liquide céphalorachidien. *Médecine et Maladies Infectieuses,* 9:306–312.

Schares G, Mehlitz D (1996). Sleeping sickness in Zaire: a nested polymerase chain reaction improves the identification of *Trypanosoma* (Trypanozoon) *brucei gambiense* by specific kinetoplast DNA probes. *Tropical Medicine and International Health*, 1:59–70.

Smith DH, Bailey JW, Wellde BT (1989). Immunodiagnostic tests on cerebrospinal fluid in the diagnosis of meningoencephalitic *Trypanosoma brucei rhodesiense* infection. *Annals of Tropical Medicine and Parasitology*, 83:91–97.

Schmid C (2004). 10-Day melarsoprol treatment of *T. b. gambiense* sleeping sickness: from efficacy to effectiveness [PhD thesis]. University of Basel, Switzerland, 2004.

Schmid C et al. (2004) Efficacy of the 10 day melarsoprol schedule two years after treatment of late-stage gambiense sleeping sickness**.** *Lancet,* 364:789–790.

Schmid C et al. (2005) Effectiveness of the 10-day melarsoprol schedule for the treatment of late-stage African trypanosomiasis: confirmation from a multi-national study (IMPAMEL II). *The Journal of Infectious Diseases*, 191:1922–1931.

Simarro PP et al. (1999). Attitude towards CATT-positive individuals without parasitological conformation in the African trypanosomiasis (*T. b. gambiense*) focus of Quiçama (Angola). *Tropical Medicine and International Health*, 4:858–861.

Simarro PP, Louis F, Jannin J (2003). *Sleeping sickness, the forgotten disease: the consequences in the field and a proposal for action.* State-of-the-Art Workshop & Round Table on Diagnosis and Treatment of HAT, 27th ISCTRC meeting, Pretoria, 2 October 2003.

Truc P et al. (1994). A comparison of parasitological methods for the diagnosis of gambian trypanosomiasis in an area of low endemicity in Côte d'Ivoire. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 88:419–421.

Truc P et al. (1998). Parasitological diagnosis of human African trypanosomiasis: a comparison of the QBC(R) and miniature anion-exchange centrifugation techniques. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 92:288–289.

Truc P et al. (2002). Evaluation of the micro-CATT, CATT/*Trypanosoma brucei gambiense*, and LATEX/*T. b. gambiense* methods for serodiagnosis and surveillance of human African trypanosomiasis in West and Central Africa. *Bulletin of the World Health Organization* 80:882–886.

Veeken HJ, Ebeling MC, Dolmans WM (1989). Trypanosomiasis in a rural hospital in Tanzania. A retrospective study of its management and the results of treatment. *Tropical and Geographical Medicine*, 41:113–117.

Welburn SC et al. (2001). Identification of human-infective trypanosomes in animal reservoir of sleeping sickness in Uganda by means of serum-resistance-associated (SRA) gene. *Lancet,* 358:2017–2019.

Whittle HC et al. (1977). IgM and antibody measurement in the diagnosis and management of Gambian trypanosomiasis. *The American Journal of Tropical Medicine and Hygiene,* 26:1129–1135.

WHO (1998) *Control and surveillance of African trypanosomiasis: report of a WHO expert committee*. Geneva, World Health Organization (WHO Technical Report Series, No. 881).

WHO (2000) Clinical study protocol: pharmacokinetic investigations on different treatment protocols for DFMO (Eflornithine). Study sponsored by WHO/TDR in Côte d'Ivoire, initiated in 2000, conducted by Dr F. Doua, Projet de Recherches Cliniques sur la Trypanosomiase PRCT, Daloa, Côte d'Ivoire.

WHO Expert Committee on Epidemiology and Control of African Trypanosomiasis (1986). *Epidemiology and control of African trypanosomiasis: report of a WHO expert committee*. Geneva, World Health Organization (WHO Technical Report Series, No. 739).

WHO/TDR and Aventis (2003). *Eflornithine investigator brochure*, UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (WHO/TDR) and Aventis.

Williamson J (1970). Review of chemotherapeutic and chemoprophylactic agents (Chapter 7). In: Mulligan HW, ed. *The African trypanosomiasis*, Allen & Unwin Ltd, London, 125–221.

Woo PTK (1971). Evaluation of the haematocrit centrifuge and other techniques for the field diagnosis of human trypanosomiasis and filariasis. *Acta Tropica,* 28:298–303.